RESPIRATORY VIRUSES in INFANCY and RAPID RSV DIAGNOSTICS

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Respiratory viruses in infancy and rapid RSV diagnostics

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Raymond Petrus (Roy) Zuurbier

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Promotor:

Prof. dr. L.J. Bont

Copromotoren:

Dr. M.A. van Houten
Dr. J.G. Wildenbeest

Beoordelingscommissie:

Prof. dr. R.A.M.J. Damoiseaux (voorzitter)
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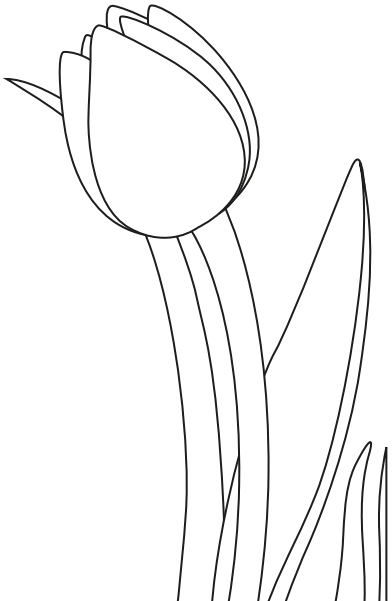
You, you were always on my mind

(Ten Sharp – You)

CONTENTS

“ ”

(Hans Zimmer – Time)



Chapter 1

General introduction

Respiratory infections

Respiratory tract infections (RTIs) are amongst the most common diseases seen in children [1,2]. They can result in lower respiratory tract infections (LRTI), such as pneumonia and bronchiolitis. With an estimated mortality of 921,000 cases in 2015, pneumonia is the second leading cause of death among young children (<5 years of age), with the highest burden in infants (<1 year of age) [1,3,4]. 99% of 'in-hospital' mortality occurs in developing countries, a figure most likely skewed due to improved access to healthcare and treatment in developed countries [4]. Due to improvement in socioeconomic circumstances, increased emphasis on preventive interventions, and improved access to and quality of healthcare, the worldwide incidence and mortality of LRTIs in young children is decreasing [5]. Nevertheless, respiratory infections are still a substantial burden on the healthcare services of developed countries.

The most frequent cause of RTIs are respiratory (seasonal) viruses [6]. Many respiratory viruses, however, can be considered pathobionts. They are frequently found in the upper respiratory tract of asymptomatic young children and can lead to both upper and lower RTIs. Well-known viral pathogens are respiratory syncytial virus (RSV), influenzavirus, and rhinovirus, the leading causes of hospitalisations, morbidity and mortality in children, especially in high-risk groups as born prematurely, having congenital heart or lung disease, or Down syndrome [4,7–9]. RSV is responsible for the majority of severe respiratory tract infections in young children [10]. However, respiratory viruses can also be found in children with mild symptoms [6,10–13]. To unravel this phenomenon, birth cohort studies are needed to include all stages of disease severity and to give a better overview of symptomatology in infants presenting with RTIs.

Most studies are largely based on hospitalised patients, focusing on the more severe cases, and only showing the tip of the iceberg. To uncover the remaining seven-eighths of the underwater iceberg and to understand the full epidemiological range of respiratory infections birth cohort or community-cohort are needed [14]. To understand the disease transmission, pathogenesis, immunity, and sequelae of respiratory viruses, studying mildly symptomatic and even asymptomatic cases is important. In addition, these non-severe patients add a substantial socio-economic burden through their misuse of antibiotics, use of healthcare services, and school and parental work absenteeism [15]. Moreover, community-based studies are needed to improve implementation of preventive interventions.

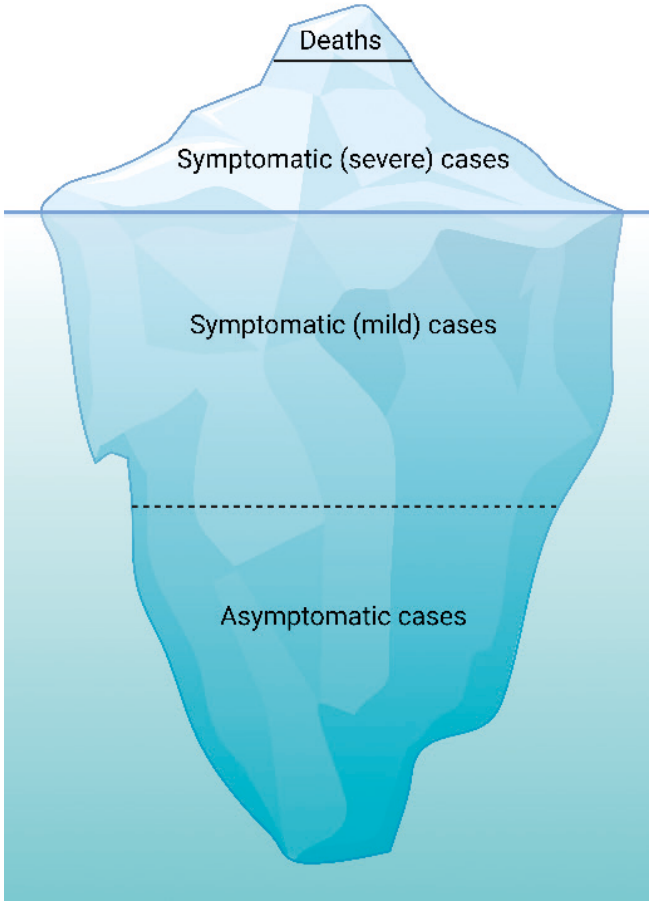


Figure 1. The iceberg of viral infections
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Respiratory syncytial virus

RSV has been isolated for the first time from chimpanzees in 1956 [16]. A year later RSV was recovered for the first time from infants presenting with bronchiolitis [17]. RSV is a single-stranded enveloped RNA virus and is family of Paramyxoviridae, genus Penumovirus [18]. It has 10 genes encoding for 11 proteins, with the G and (F) fusion glycoproteins on the virus' surface. The G protein is for host cell attachment, the F protein is for fusion and cell entry. In 2013 it was discovered that F protein has a prefusion and postfusion state, which is important as most vaccine candidates are targeting F protein [19–21]. Based on differences in G protein there are two antigenic variants: RSV type A and B. Both types circulate during generally during winter season [22], however during the COVID-19 pandemic delayed RSV outbreaks were observed

[23]. This was probably due to social distancing and other lockdown strategies, slowing down the spread of respiratory viruses [24]. Reduced exposure leads to waning of immunity of RSV and could therefore lead to an extensive outbreak.

RSV has an incubation period of approximately 5 days (range: two to eight days). It generally starts with symptoms of an upper RTI, i.e. rhinorrhea or nasal congestion and coughing [25]. RSV can progress to the lower respiratory tract in 3-5 days by inflaming the bronchioles and leading to bronchiolitis. Bronchiolitis can cause tachypnea, wheezing, fever and dyspnea which can result in reduced oral intake [26,27]. A severe symptom is apnea, occurring in approximately 5% of the cases, with an increased risk for preterm born infants [27]. Main reasons for hospital admission in children with RSV bronchiolitis are hypoxia or respiratory failure, reduced feeding, and dehydration [28]. So far, treatment for severe RSV is mainly supportive by respiratory support and supplemental oxygen, and feeding by nasogastric tube.

RSV causes severe disease in individuals at the extremes of the age spectrum, in high-risk groups, and is the most common cause of bronchiolitis and pneumonia in infants [8]. Of the young children, infants represent 75% of RSV hospitalisations [29,30]. Well-known risk factors for severe disease in young children are prematurity and cardiorespiratory comorbidity, however, a substantial number of children admitted to the hospital due to a severe RSV infection are previously healthy, full-term infants [29,30]. In 2015 it was estimated that RSV was associated with 33.1 million cases of LRTI, 3.2 million LRTI hospitalizations, and 48,000 to 74,500 deaths in children <5 years, worldwide [8].

RSV infection also occurs in adults and are responsible for a significant burden [31–33]. Although, RSV infections in adults are often milder, they can still cause severe respiratory disease. Especially older adults and those with immunodeficiency or cardiopulmonary comorbidities are the most vulnerable for severe RSV infection [31,33,34].In these patients the burden is similar to that of non-pandemic influenza and can result in LRTI, and even death. The overall annual incidence of RSV is estimated at 3-7% in healthy older adults, but rarely causes severe disease[31,35,36]. About 45% of hospital admissions due to RSV-LRTI occur in children younger than 6 months [8]. To date, treatment and prophylaxis options are limited. For patients with severe RSV-LRTI, often only supportive care is available in the form of supplemental oxygen, or mechanical ventilation. Palivizumab (Synagis®), an RSV RTI prophylactic,

is a monoclonal antibody relying upon a passive administration strategy to prevent severe RSV infection. It has to be administered monthly by intramuscular injection, is very expensive, and has a number needed to treat around 20 to prevent one RSV-related hospitalisation in high-risk groups [37]. An effective monoclonal antibody or maternal RSV vaccine could have a substantial effect on disease burden in this age group. The RSV vaccine development has been revolutionised by advances in the field of RSV surface fusion (F) glycoprotein structural biology [21]. Highly potent monoclonal antibodies, such as Nirsevimab, with an extended half-life are showing promising results [38]. It has demonstrated protection against RSV by significantly reducing the number of (in- or outpatient) previously healthy preterm [38] and full-term infants [39] with LRTIs requiring medical attention. Nirsevimab reduced the incidence of hospitalisation for RSV-associated LRTI in preterm infants with 78.4% [38]. Another strategy to reduce the incidence of RSV infections is maternal vaccination, protecting infants in their first months of life through passive immunization. This strategy is already widely implemented for the prevention of influenza and Bordetella pertussis and since 2021 also for SARS-CoV-2 [40–42]. In the Netherlands the uptake for maternal Bordetella pertussis vaccination was estimated at 70% in 2020.

Also novel therapeutics such as antivirals are in development [43,44], with some already in phase 3 [45,46]. To be most effective they have to be administered in an early stage of the infection to prevent the development of severe disease. Therefore rapid diagnosis of RSV is needed, especially testing in an early stage, for instance by the general practitioner.

Diagnostics

Currently, the gold standard for RSV diagnosis is laboratory based reverse transcriptase polymerase chain reaction (RT-PCR). This technique has a high sensitivity and specificity, but is time-consuming, relies on trained laboratory staff, and has a significant delay of 24-48 hours before results are available for clinical teams, negating its clinical value. Reliable rapid diagnostic tests would also decrease the prescription of unnecessary antibiotics [47,48] and enable cohorting of hospitalised patients in the RSV season. An evolving role for rapid tests is as a companion diagnostic tool for the development and use of novel RSV antivirals [43], and for the evaluation of efficacy of new RSV vaccines, both for which a rapid and reliable and RSV test will be critical.

In recent years several point-of-care tests (POCTs) have been developed to detect RSV, such as rapid antigen tests (RADTs) and molecular assays. A range of POCTs are available and already used in clinical practice since they are fast, easy to use, and often less expensive than a routine RT-PCR. RSV rapid antigen detection tests (RADTs) are POCTs with high specificity, but a wide range in sensitivity, partially depending on viral load [49,50]. Most often used technique in RADTs is lateral-flow immunochromatographic assays. They can be compared with pharmacy pregnancy tests. The lateral flow test uses capillary flow to carry the analyte first trough antibodies conjugated tag. Then it migrates further, where it binds a second set of virus-specific antibodies, producing a coloured result line, indicating a positive test [50]. Two recent meta-analyses showed a pooled sensitivity of 81% (95% CI, 78-84%) [51] and 75.9% (95% CI, 73.1-78.5%) [52] for RSV RADTs in children compared to RT-PCR. There is a large heterogeneity in these studies, which are often sponsored by the tests' manufacturer. In addition, many studies are performed retrospectively and in hospitalised children, and the diagnostics are not evaluated at point-of-care. As a result, sensitivity of individual studies varies considerably from 41.2% [53] to 83%[54].

PCR-based molecular assays are also available and are used in clinical practice because they are fast, easy to use by non-laboratory personnel, and often less expensive compared to routine RT-PCR. The turnaround time of most molecular POCTs is less than an hour. The use of molecular POCTs is associated with a significant reduction in hospital length of stay, testing costs, and isolation time [55,56]. The Xpert® Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA) and the ID NOW™ RSV assay (Abbott Diagnostics Scarborough, Inc., Scarborough, ME) are two molecular POCTs commercially available [57]. ID NOW™, previously known as Alere i, RSV assay, is based on nicking enzyme amplification reaction (NEAR), making low-complex PCR-based amplification possible as it uses constant low temperatures. The Xpert® Xpress Flu/RSV assay technology has fully-automated steps of extraction, amplification and detection. Studies report a high sensitivity and specificity, however, these bear the risk of overestimating test accuracy. They were performed in medically attended or hospitalized patients, used remnant specimen samples, were partially performed in children with predictably high viral loads, were more often than not sponsored by the manufacturer, and were performed in relatively small numbers of patients. Therefore, it is important to evaluate test performance in community based cohorts, to include different degrees of disease severity.

Influenza

In addition to RSV, influenza is also recognized as major pathogen causing acute lower respiratory infection (ALRI) [10]. In human it is mostly caused by type A or B influenzaviruses, both related to Orthomyxoviridae family. These RNA viruses cause winter-seasonal epidemics in the northern hemisphere each winter [58]. Influenza has long been identified as a disease of the elderly, sparing almost all but the high-risk group of children [59]. However, recent studies suggest that the burden of disease in young children is also substantial [60–62]. Influenza in young children (<5 years) is detected in 3-7% of the ALRI admissions [9,63]. It was estimated that influenza was associated with 10.1 million cases of LRTI, 870,000 LRTI hospitalizations, 15,300 in-hospital deaths, and up to 34,800 overall influenza virus-associated ALRI deaths in young children in 2018 worldwide [61]. The majority of in-hospital deaths occurred in low-income, and lower-middle-income countries [61]. Furthermore, it was estimated that outpatient visits due to influenza were 10 to 250 times as common as hospitalizations [64], showing the importance of community-based data when estimating disease burden. The burden of influenza among young children (<5 y) increases with age, among infants the highest burden of influenza is in children aged 6-11 months [9,61]. Nevertheless, among young children about 23% of the hospital admissions and 36% of the in-hospital deaths were infants under 6 months [61].

Treatment of influenza infection is mostly supportive. Oseltamivir is an antiviral drug which can be administered in an early stage to shorten the duration an severity of symptoms [65,66]. In the Netherlands only high-risk groups are vaccinated against influenza. Influenza vaccines are licensed for children older than 6 months [67]. To bridge the gap, maternal vaccination is a safe and effective intervention to protect infants during their first months of life [68]. Through placental transfer of maternal antibodies, infants will be protected against influenza. To protect mothers as well as their infants, in 2012, the World Health Organization (WHO) recommended the influenza vaccination for pregnant women. Despite this recommendation, the global maternal vaccination rate for influenza remains low [68].

Although RSV is the most prevalent pathogen in children with ALRI [69], an increasing number of publications suggest that the burden on healthcare worldwide due to influenza in young children is still substantial [9,62]. Opposed to influenza, the burden of RSV is expected to be highest among infants as most RSV-related mortalities occur during the first year of life [70]. Conversely, most attention about influenza has

been drawn to the impact of the virus in the elderly because of its high incidence, hospitalisation, and mortality rates [71]. Little is known about which of the two viruses is more pathogenic and if there are differences between age groups. A broad comparison between the burdens of both respiratory virus infections, particularly among previously healthy infants, can help in bringing more information about immunisation recommendations and prioritise research and development funding for the future. To answer these questions large birth cohorts are needed.

Rhinovirus

Rhinoviruses (RV) are the most prevalent respiratory virus in infants, however detected more often in controls (infants without symptoms) than infants with respiratory symptoms [12,72,73]. Almost all infants develop at least one RV infection in the first year of life [74]. Rhinovirus belongs to the order of Picornavirales, family Picornaviridae and genus Enterovirus. There are over 160 genotypes and serotypes and are classified into three RV species; A, B and C [75]. RV consists of a simple viral capsid and a single RNA strand [76,77]. Clinical presentations in infants with RV are ranging from asymptomatic to severe infections requiring hospilisation [72]. Yet, RV is often detected as causal agent of bronchiolitis in hospitalised infants. However, the role of RV can been overestimated, mainly due to non-controlled designs of studies [78]. It is suggested that severe symptoms due to RV infections are linked to respiratory susceptibility of the host [73]. Regarding the association of recurrent wheezing and lower respiratory tract infections, RV has a comparable or even more important role in this association [79]. Underlining the suggestion of severe RV infection reserved for those who are susceptible to respiratory morbidity, also a high number of infants only have mild symptoms. This variation in clinical presentation and the pathogenesis are still subject of ongoing research and need to be studied more in detail.

Sequelae of respiratory viruses

Early life RSV and rhinovirus-associated LRTIs have been suggested to play an important role in the development of recurrent wheezing, asthma, and possibly allergic sensitisation later in life [81–86]. These long-term sequelae pose a substantial, additional burden upon the healthcare system [87]. However, it is unclear whether these LRTIs are a causal factor or a manifestation of a predisposition to other respiratory illnesses. This is crucial information for policy makers responsible for the prevention of LRTIs.

A large meta-analysis including more than 40 studies estimating the association between RSV infection in childhood and subsequent wheezing and asthma has been done [79]. While not necessarily indicating a causal relationship, it does show an association between early life RSV infection and subsequent childhood recurrent wheeze. This association was only found when comparing early life RSV infection to those who were healthy or those without respiratory symptoms. The association was not significant for RSV versus any other pathogen, and even negatively associated when compared to children with a rhinovirus infection. This suggests that rhinovirus could play a comparable, or more important role in the development of recurrent wheezing.

Rhinovirus associated LRTI during the first year of life is also associated with wheezing, however the underlying mechanism remains unclear, and most studies are based on hospitalized infants [6,72]. What is known is that also asymptomatic presence of rhinovirus is suggested to be a potent activator of the airway mucosal immune system, with predominant enhancement of type 1 proinflammatory mediators, which may be predictive of future asthma development and allergic sensitisation [88].

It is suggested that there is a critical window during infancy when environmental exposures, and especially acute RSV and rhinovirus infections, may shape the remodelling of the airway and the functioning of a developing immune system. This could explain the association between these viral infections in early life and the subsequent development of recurrent wheezing an asthma. The immune response, including weaker interferon responses, evolves during infancy. Young infants lack immunologic memory, and have a biased tolerogenic immune response (Tregs & Th2 responses), along with a restrained Th1 immunity associated with disease severity. These specific nuances in the immune response may explain the infant's susceptibility to these infections and their association with the development of recurrent wheezing/asthma later in life [89].

OUTLINE OF THIS THESIS

The aim of this thesis is to examine the accuracy of different RSV rapid tests and, dynamics and burden of respiratory viruses in a community setting, including different levels of severity of respiratory infections.

Specific research questions addressed in this thesis:

Chapter 2: What is the performance of the RADT BinaxNOW RSV to diagnose RSV infection in infants with acute respiratory tract infection (ARTI) in different clinical settings?

Chapter 3: Is the performance of the Xpert® Xpress Flu/RSV assay comparable to RT-PCR to diagnose RSV infection in home-dwelling older adults (≥60 years) with ARTI in different clinical settings?

Chapter 4: What is the prevalence of viruses across the first year of life, and its relationship with acute and subsequent RTI symptoms in the first year of life?

Chapter 5: How will we provide key information to fill the gaps in knowledge about the burden of RSV disease in healthy infants?

Chapter 6: What is the burden of RSV disease in healthy infants?

Chapter 7: What is the burden of influenza compared to RSV infection in community-dwelling and hospitalised infants?

This thesis is divided into two parts. In the first part, we focus on point-of-care testing on RSV. The second part is about respiratory viruses found in infancy.

Part one: RSV point-of-care testing

Part one describes two studies evaluating the performance of RSV POCTs in infants (Chapter 2) and older adults (Chapter 3) with ARTI in different clinical settings. Both studies are community-based, allowing us to evaluate the performance of RSV POCTs on different levels of ARTI severity. In **Chapter 2** we describe the performance of the RADT BinaxNOW RSV in our RESCEU birth cohort study compared with a molecular-based assay. **Chapter 3** describes the performance of a molecular-based POCT called Xpert® Xpress Flu/RSV assay. This study includes home-dwelling older adults with ARTI and evaluates the comparability of this POCT with RT-PCR to diagnose RSV infection in different clinical settings.

Part two: Respiratory viruses in infants

In part two results on respiratory viruses in infants from two different birth cohorts are described: The MUIS birth cohort and RESCEU birth cohort. Both cohorts were are from the Spaarne Gasthuis. The MUIS birth cohort aims to investigate the development of the infants microbiome. Nasopharyngeal swabs were obtained at from 11 consecutive regular sampling moments, and during acute RTIs across the first year of life. In **Chapter 4** these swabs were tested on a panel of 17 respiratory viruses and we describe the prevalence of viruses across the first year of life, and its relationship with acute and subsequent RTI symptoms in the first year of life. The RESCEU birth cohort study is an international study and aims to determine the incidence of RSV infection-associated ARTI, RSV-associated medically attended ARTI, and RSV-related hospitalisation during the first year of life. **Chapter 5** describes the objectives and methods of this study. The study consists approximately 10,000 healthy infants recruited during 3 consecutive years, from the general population. 1,000 infants were actively followed. In case of ARTI, a respiratory sample was collected for RSV molecular diagnosis. In **Chapter 6** we describe the findings of this study, by evaluating the burden of RSV in healthy term born infants in Europe. **Chapter 7** evaluates the burden of influenza versus RSV of the Dutch part of the RESCEU cohort and a hospital cohort executed at the Spaarne Gasthuis. **Chapter 8** provides a general discussion, summarising the main findings of this thesis, and discusses the clinical implications and future perspectives of part one on RSV point-of-care testing.

REFERENCES

1. Liu L, Oza S, Hogan D, *et al.* Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016; **388**: 3027–35.
2. Troeger C, Forouzanfar M, Rao PC, *et al.* Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis* 2017; **17**: 1133–61.
3. Vos T, Lim SS, Abbafati C, *et al.* Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020; **396**: 1204–22.
4. Nair H, Simões EA, Rudan I, *et al.* Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. *Lancet* 2013; **381**: 1380–90.
5. McAllister DA, Liu L, Shi T, *et al.* Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. *Lancet Glob Heal* 2019; **7**: e47–57.
6. Kusel MMH, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of Respiratory Viruses in Acute Upper and Lower Respiratory Tract Illness in the First Year of Life. *Pediatr Infect Dis J* 2006; **25**: 680–6.
7. Wang X, Li Y, O'Brien KL, *et al.* Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. *Lancet Glob Heal* 2020; **8**: e497–510.
8. Shi T, McAllister DA, O'Brien KL, *et al.* Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 2017; **390**: 946–58.
9. Lafond KE, Nair H, Rasooly MH, *et al.* Global Role and Burden of Influenza in Pediatric Respiratory Hospitalizations, 1982–2012: A Systematic Analysis. *PLOS Med* 2016; **13**: e1001977.
10. Shi T, McLean K, Campbell H, Nair H. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta–analysis. *J Glob Health* 2015; **5**. DOI:10.7189/jogh.05.010408.
11. Sarna M, Lambert SB, Sloots TP, *et al.* Viruses causing lower respiratory symptoms in young children: findings from the ORChID birth cohort. *Thorax* 2018; **73**: 969–79.
12. van der Zalm MM, Uiterwaal CSPM, Wilbrink B, *et al.* Respiratory Pathogens in Respiratory Tract Illnesses During the First Year of Life. *Pediatr Infect Dis J* 2009; **28**: 472–6.
13. Singleton RJ, Bulkow LR, Miernyk K, *et al.* Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol* 2010; **82**: 1282–90.
14. Nair H. Role of community-based cohorts for uncovering the iceberg of disease. *Lancet Glob Heal* 2021; **9**: e740–1.
15. Heikkinen T, Ojala E, Waris M. Clinical and Socioeconomic Burden of Respiratory Syncytial Virus Infection in Children. *J Infect Dis* 2017; **215**: 17–23.
16. Morris JA, Blount RE, Savage RE. Recovery of Cytopathogenic Agent from Chimpanzees with Goryza. *Exp Biol Med* 1956; **92**: 544–9.

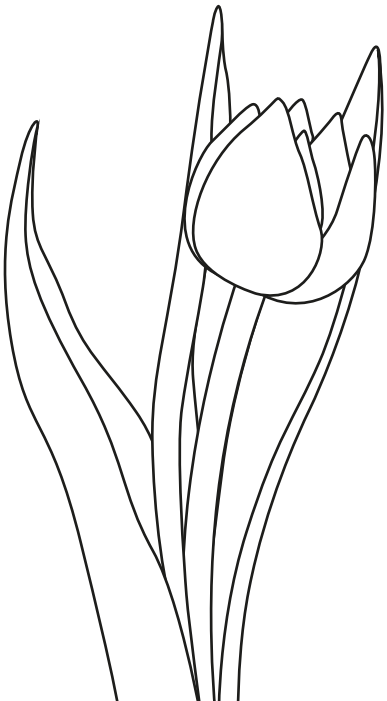
17. CHANOCK R, ROIZMAN B, MYERS R. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation, properties and characterization. *Am J Hyg* 1957; **66**: 281–90.
18. Borchers AT, Chang C, Gershwin ME, Gershwin LJ. Respiratory syncytial virus - A comprehensive review. Clin. Rev. Allergy Immunol. 2013; **45**: 331–79.
19. McLellan JS, Chen M, Joyce MG, *et al.* Structure-Based Design of a Fusion Glycoprotein Vaccine for Respiratory Syncytial Virus. *Science (80-)* 2013; **342**: 592–8.
20. McLellan JS, Chen M, Leung S, *et al.* Structure of RSV Fusion Glycoprotein Trimer Bound to a Prefusion-Specific Neutralizing Antibody. *Science (80-)* 2013; **340**: 1113–7.
21. Mazur NI, Higgins D, Nunes MC, *et al.* The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. Lancet Infect. Dis. 2018; **18**: e295–311.
22. Hall CB, Walsh EE, Schnabel KC, *et al.* Occurrence of Groups A and B of Respiratory Syncytial Virus over 15 Years: Associated Epidemiologic and Clinical Characteristics in Hospitalized and Ambulatory Children. 1990 https://www-jstor-org.proxy.library.uu.nl/stable/pdf/30127900.pdf?refreqid=excelsior%3A0a19db3b22c041f12fd776e7456fffea (accessed Feb 12, 2019).
23. Delestrain C, Danis K, Hau I, *et al.* Impact of COVID-19 social distancing on viral infection in France: A delayed outbreak of RSV. *Pediatr Pulmonol* 2021; : ppul.25644.
24. Kuitunen I, Artama M, Mäkelä L, Backman K, Heiskanen-Kosma T, Renko M. Effect of Social Distancing Due to the COVID-19 Pandemic on the Incidence of Viral Respiratory Tract Infections in Children in Finland during Early 2020. *Pediatr Infect Dis J* 2020; **39**: E423–7.
25. Ricci V, Delgado Nunes V, Murphy MS, Cunningham S. Bronchiolitis in children: summary of NICE guidance. *BMJ* 2015; **350**: h2305–h2305.
26. Smyth RL, Openshaw PJ. Bronchiolitis. *Lancet* 2006; **368**: 312–22.
27. Fretzayas A, Moustaki M. Etiology and clinical features of viral bronchiolitis in infancy. *World J Pediatr* 2017; **13**: 293–9.
28. Coultas JA, Smyth R, Openshaw PJ. Respiratory syncytial virus (RSV): a scourge from infancy to old age. *Thorax* 2019; **74**: 986–93.
29. Hall CB, Weinberg GA, Blumkin AK, *et al.* Respiratory Syncytial Virus-Associated Hospitalizations Among Children Less Than 24 Months of Age. *Pediatrics* 2013; **132**: e341–8.
30. Hall CB, Weinberg GA, Iwane MK, *et al.* The Burden of Respiratory Syncytial Virus Infection in Young Children. *N Engl J Med* 2009; **360**: 588–98.
31. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory Syncytial Virus Infection in Elderly and High-Risk Adults. *N Engl J Med* 2005; **352**: 1749–59.
32. Shi T, Denouel A, Tietjen AK, *et al.* Global Disease Burden Estimates of Respiratory Syncytial Virus–Associated Acute Respiratory Infection in Older Adults in 2015: A Systematic Review and Meta-Analysis. *J Infect Dis* 2020; **222**: S577–83.
33. Falsey AR, Walsh EE, Esser MT, Shoemaker K, Yu L, Griffin MP. Respiratory syncytial virus–associated illness in adults with advanced chronic obstructive pulmonary disease and/or congestive heart failure. *J Med Virol* 2019; **91**: 65–71.
34. Ackerson B, Tseng HF, Sy LS, *et al.* Severe Morbidity and Mortality Associated With Respiratory Syncytial Virus Versus Influenza Infection in Hospitalized Older Adults. *Clin Infect Dis* 2019; **69**: 197–203.
35. Korsten K, Adriaenssens N, Coenen S, *et al.* Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study. *Eur Respir J* 2021; **57**: 2002688.

36. Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. *BMJ* 1997; **315**: 1060–4.
37. Andabaka T, Nickerson JW, Rojas-Reyes MX, Rueda JD, Bacic Vrca V, Barsic B. Monoclonal antibody for reducing the risk of respiratory syncytial virus infection in children. *Cochrane Database Syst Rev* 2013; **8**: 2377–9.
38. Griffin MP, Yuan Y, Takas T, *et al.* Single-Dose Nirsevimab for Prevention of RSV in Preterm Infants. *N Engl J Med* 2020; **383**: 415–25.
39. Muller W. Nirsevimab demonstrated protection against respiratory syncytial virus disease in healthy infants in Phase 3 trial. .
40. WHO. Vaccines against influenza WHO position paper – November 2012. *Wkly Epidemiol Rec* 2012; **87**: 461–76.
41. WHO. Pertussis vaccines: WHO position paper, August 2015—Recommendations. *Vaccine* 2016; **34**: 1423–5.
42. Shimabukuro TT, Kim SY, Myers TR, *et al.* Preliminary Findings of mRNA Covid-19 Vaccine Safety in Pregnant Persons. *N Engl J Med* 2021; **384**: 2273–82.
43. Mazur NI, Martinón-Torres F, Baraldi E, *et al.* Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. *Lancet Respir Med* 2015; **3**: 888–900.
44. Cunningham S, Piedra PA, Martinon-Torres F, *et al.* Nebulised ALX-0171 for respiratory syncytial virus lower respiratory tract infection in hospitalised children: a double-blind, randomised, placebo-controlled, phase 2b trial. *Lancet Respir Med* 2021; **9**: 21–32.
45. A Study of Rilematovir in Infants and Children and Subsequently in Neonates Hospitalized With Acute Respiratory Tract Infection Due to Respiratory Syncytial Virus (RSV) - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04583280 (accessed Aug 5, 2021).
46. Jorquera PA, Tripp RA. Respiratory syncytial virus: prospects for new and emerging therapeutics. *Expert Rev Respir Med* 2017; **11**: 609–15.
47. van Houten CB, Naaktgeboren C, Buiteman BJM, *et al.* Antibiotic Overuse in Children with Respiratory Syncytial Virus Lower Respiratory Tract Infection. *Pediatr Infect Dis J* 2018; **37**: 1077–81.
48. van Houten CB, Cohen A, Engelhard D, *et al.* Antibiotic misuse in respiratory tract infections in children and adults—a prospective, multicentre study (TAILORED Treatment). *Eur J Clin Microbiol Infect Dis* 2019; **38**: 505–14.
49. Miernyk K, Bulkow L, Debyle C, *et al.* Performance of a rapid antigen test (Binax NOW ® RSV) for diagnosis of respiratory syncytial virus compared with real-time polymerase chain reaction in a pediatric population. *J Clin Virol* 2010; **50**: 240–3.
50. Prendergast C, Papenburg J. Rapid antigen-based testing for respiratory syncytial virus: moving diagnostics from bench to bedside? *Future Microbiol* 2013; **8**: 435–44.
51. Chartrand C, Tremblay N, Renaud C, Papenburg J. Diagnostic Accuracy of Rapid Antigen Detection Tests for Respiratory Syncytial Virus Infection: Systematic Review and Meta-analysis. *J Clin Microbiol* 2015; **53**: 3738–49.
52. Bruning AHL, Leeflang MMG, Vos JMBW, *et al.* Rapid Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2017; **65**: 1026–32.
53. Khanom AB, Velvin C, Hawrami K, *et al.* Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. *J Infect* 2011; **62**: 52–8.

54. Mills JM, Harper J, Broomfield D, Templeton KE. Rapid testing for respiratory syncytial virus in a paediatric emergency department: benefits for infection control and bed management. *J Hosp Infect* 2011; **77**: 248–51.
55. Wabe N, Li L, Lindeman R, *et al.* Impact of Rapid Molecular Diagnostic Testing of Respiratory Viruses on Outcomes of Adults Hospitalized with Respiratory Illness: a Multicenter Quasi-experimental Study. *J Clin Microbiol* 2018; **57**. DOI:10.1128/JCM.01727-18.
56. Rogers BB, Shankar P, Jerris RC, *et al.* Impact of a Rapid Respiratory Panel Test on Patient Outcomes. *Arch Pathol Lab Med* 2015; **139**: 636–41.
57. A. Hogan C, Caya C, Papenburg J. Rapid and simple molecular tests for the detection of respiratory syncytial virus: a review. *Expert Rev Mol Diagn* 2018; **18**: 617–29.
58. Prevention and control of influenza pandemics and annual epidemics. 2003.
59. Iskander M, Booy R, Lambert S. The burden of influenza in children. Curr. Opin. Infect. Dis. 2007; **20**: 259–63.
60. Poehling KA, Edwards KM, Griffin MR, *et al.* The Burden of Influenza in Young Children, 2004-2009. *Pediatrics* 2013; **131**: 207–16.
61. Wang X, Li Y, O'Brien KL, *et al.* Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. *Lancet Glob Heal* 2020; **8**: e497–510.
62. Nair H, Brooks WA, Katz M, *et al.* Global burden of respiratory infections due to seasonal influenza in young children: A systematic review and meta-analysis. *Lancet* 2011; **378**: 1917–30.
63. Lukšić I, Kearns PK, Scott F, Rudan I, Campbell H, Nair H. Viral etiology of hospitalized acute lower respiratory infections in children under 5 years of age – a systematic review and meta-analysis. *Croat Med J* 2013; **54**: 122–34.
64. Poehling KA, Edwards KM, Weinberg GA, *et al.* The Underrecognized Burden of Influenza in Young Children. *N Engl J Med* 2006; **355**: 31–40.
65. Mattila J, Vuorinen T, Waris M, Antikainen P, Heikkinen T. Oseltamivir treatment of influenza A and B infections in infants. *Influenza Other Respi Viruses* 2021; : irv.12862.
66. Butler CC, van der Velden AW, Bongard E, *et al.* Oseltamivir plus usual care versus usual care for influenza-like illness in primary care: an open-label, pragmatic, randomised controlled trial. *Lancet* 2020; **395**: 42–52.
67. Grohskopf LA, Alyanak E, Broder KR, Walter EB, Fry AM, Jernigan DB. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2019–20 Influenza Season. *MMWR Recomm Reports* 2019; **68**: 1–21.
68. Anderson LJ, Dormitzer PR, Nokes DJ, Rappuoli R, Roca A, Graham BS. Strategic priorities for respiratory syncytial virus (RSV) vaccine development. *Vaccine* 2013; **31 Suppl 2**: B209-15.
69. Nair H, Nokes DJ, Gessner BD, *et al.* Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010; **375**: 1545–55.
70. Scheltema N. Respiratory syncytial virus-related disease burden in young children.
71. Nichol KL. Influenza vaccination in the elderly: Impact on hospitalisation and mortality. Drugs and Aging. 2005; **22**: 495–515.
72. Kieninger E, Fuchs O, Latzin P, Frey U, Regamey N. Rhinovirus infections in infancy and early childhood. Eur. Respir. J. 2013; **41**: 443–52.
73. Mack I, Kieninger E, Cangiano G, *et al.* Rhinovirus Infections and Associated Respiratory Morbidity in Infants. *Pediatr Infect Dis J* 2016; **35**: 1069–74.

74. van der Zalm MM, Wilbrink B, van Ewijk BE, Overduin P, Wolfs TFW, van der Ent CK. Highly frequent infections with human rhinovirus in healthy young children: A longitudinal cohort study. *J Clin Virol* 2011; **52**: 317–20.
75. Jacobs SE, Lamson DM, St. George K, Walsh TJ. Human Rhinoviruses. *Clin Microbiol Rev* 2013; **26**: 135–62.
76. Royston L, Tapparel C. Rhinoviruses and Respiratory Enteroviruses: Not as Simple as ABC. *Viruses* 2016; **8**: 16.
77. Palmenberg AC, Spiro D, Kuzmickas R, *et al*. Sequencing and Analyses of All Known Human Rhinovirus Genomes Reveal Structure and Evolution. *Science (80-)* 2009; **324**: 55–9.
78. Spichak T V., Yatsyshina SB, Katosova L, Kim SS, Korppi MO. Is the role of rhinoviruses as causative agents of pediatric community-acquired pneumonia over-estimated? *Eur J Pediatr* 2016; **175**: 1951–8.
79. Shi T, Ooi Y, Zaw EM, *et al*. Association Between Respiratory Syncytial Virus-Associated Acute Lower Respiratory Infection in Early Life and Recurrent Wheeze and Asthma in Later Childhood. *J Infect Dis* 2020; **222**: S628–33.
80. Williams GR, Kubajewska I, Glanville N, Johnston SL, Mclean GR. The potential for a protective vaccine for rhinovirus infections. *Expert Rev Vaccines* 2016; **15**: 569–71.
81. Rubner FJ, Jackson DJ, Evans MD, *et al*. Early life rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence. *J Allergy Clin Immunol* 2017; **139**: 501–7.
82. Scheltema NM, Nibbelke EE, Pouw J, *et al*. Respiratory syncytial virus prevention and asthma in healthy preterm infants: a randomised controlled trial. *Lancet Respir Med* 2018; **2600**: 1–8.
83. Brunwasser SM, Snyder BM, Driscoll AJ, *et al*. Assessing the strength of evidence for a causal effect of respiratory syncytial virus lower respiratory tract infections on subsequent wheezing illness: a systematic review and meta-analysis. *Lancet Respir Med* 2020; **8**: 795–806.
84. Moraes TJ, Sears MR. Lower respiratory infections in early life are linked to later asthma. *Thorax* 2018; **73**: 105–6.
85. Hasegawa K, Mansbach JM, Bochkov YA, *et al*. Association of Rhinovirus C Bronchiolitis and Immunoglobulin E Sensitization During Infancy With Development of Recurrent Wheeze. *JAMA Pediatr* 2019; **1101**: 1–9.
86. Liu L, Pan Y, Zhu Y, *et al*. Association between rhinovirus wheezing illness and the development of childhood asthma: a meta-analysis. *BMJ Open* 2017; **7**: 13034.
87. Asher I, Pearce N. Global burden of asthma among children. *Int J Tuberc Lung Dis* 2014; **18**: 1269–78.
88. Wolsk HM, Følsgaard N V, Birch S, *et al*. Picornavirus-Induced Airway Mucosa Immune Profile in Asymptomatic Neonates. *J Infect Dis* 2016; **213**: 1262–70.
89. Heinonen S, Rodriguez-Fernandez R, Diaz A, Oliva Rodriguez-Pastor S, Ramilo O, Mejias A. Infant Immune Response to Respiratory Viral Infections. *Immunol Allergy Clin North Am* 2019; **39**: 361–76.

I can still hear you saying
We would never break the chain
(Fleetwood Mac – The Chain)



Chapter 2

Low sensitivity of BinaxNOW® RSV in infants

Roy P. Zuurbier, Louis J. Bont, Annefleur C. Langedijk, Mirjam Hamer, Koos Korsten, Simon B. Drysdale, Matthew D. Snape, Hannah Robinson, Andrew J. Pollard, Federico Martinón-Torres, Carmen Rodríguez-Tenreiro Sánchez, Alberto Gómez-Carballa, Ana Isabel Dacosta-Urbieta,Terho Heikkinen, Steve Cunningham, Marlies A. van Houten, and Joanne G. Wildenbeest; for the RESCEU Investigators

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ABSTRACT

Background. Respiratory syncytial virus (RSV) is a major cause of hospitalization in infants. Early detection of RSV can optimize clinical management and minimize use of antibiotics. BinaxNOW RSV (BN) is a rapid antigen detection test that is widely used. We aimed to validate the sensitivity of BN in hospitalized and nonhospitalized infants against the gold standard of molecular diagnosis.

Methods. We evaluated the performance of BN in infants with acute respiratory tract infections with different degrees of disease severity. Diagnostic accuracy of BN test results were compared with molecular diagnosis as reference standard.

Results. One hundred sixty-two respiratory samples from 148 children from October 2017 to February 2019 were studied. Sixty-six (40.7%) samples tested positive for RSV (30 hospitalizations, 31 medically attended episodes not requiring hospitalization, and 5 nonmedically attended episodes). Five of these samples tested positive with BN, leading to an overall sensitivity of BN of 7.6% (95% confidence interval [CI], 3.3%–16.5%) and a specificity of 100% (95% CI, 96.2%–100%). Sensitivity was low in all subgroups.

Conclusions. We found a low sensitivity of BN for point-of-care detection of RSV infection. BinaxNOW RSV should be used and interpreted with caution.

Clinical Trials Registration: NCT03627572, NCT03756766

Keywords

antigen detection; birth cohort; diagnosis; point-of-care test; respiratory syncytial virus.

BACKGROUND

Respiratory syncytial virus (RSV) is the most common pathogen identified in young children with acute lower respiratory tract infections [1]. Respiratory syncytial virus is a major cause of hospital admissions with an estimated hospitalization rate of 19 per 1000 children under the age of 1 year worldwide [2–4].

Reliable rapid diagnostic tests are needed to improve patient management regarding unnecessary use of antibiotics [5, 6] and to enable cohorting of hospitalized children in the RSV season. An evolving role for rapid tests is as a companion diagnostic for the development of novel RSV antivirals and evaluation of efficacy of new RSV vaccines, for which it will be important to have both a reliable and rapid RSV test.

The current gold standard for RSV diagnosis is laboratory-based reverse-transcriptase polymerase chain reaction (RT-PCR). This technique is highly sensitive and specific, but it is time-consuming, relies on trained laboratory staff, and typically has a long lag time to provide results to clinical teams (24–48 hours), negating its clinical value. Although in recent years point-of-care tests (POCTs) utilizing molecular methods have been developed, they remain expensive and consequently are not widely adopted in clinical practice. A range of alternative POCTs are available and used in clinical practice that are fast, easy to use by nonlaboratory personnel, and often less expensive compared with routine RT-PCR. The turnaround time of most POCTs is less than 1 hour. Respiratory syncytial virus rapid antigen detection tests (RADTs) are POCTs with high specificity, but a wide range in sensitivity, partially depending on viral load [7, 8]. Two recent meta-analyses showed a pooled sensitivity of 81% (95% confidence interval [CI], 78%–84%) [9] and 75.9% (95% CI, 73.1%–78.5%) for RSV RADTs in general in children compared with RT-PCR [10]. There is large heterogeneity in these studies, which are often sponsored by the tests' manufacturer. In addition, many studies are performed retrospectively and in hospitalized children, whereas diagnostics are not evaluated at point of care (POC). As a result, sensitivity of individual studies vary considerably from 41.2% [11] to 83% [12].

The aim of the current study was to evaluate for the first time the performance of the RADT BinaxNOW RSV ([BN] Alere Inc., Waltham, MA) [13] to diagnose RSV infection in infants with acute respiratory tract infection (ARTI) in different clinical settings in a large international prospective clinical study.

METHODS

Study population

The study population consisted of infants (<1 year old) with an ARTI who were participating in the REspiratory Syncytial virus Consortium in EUrope (RESCEU) [14] birth cohort study or the case-control study during 2 RSV seasons between 1 October 2017 and 28 February 2019. The RESCEU is a European Union-funded consortium study aiming to define RSV burden of disease in Europe. The current study was performed in the Netherlands, Spain and the United Kingdom. The birth cohort study consists of healthy infants prospective followed up from birth. In their first year of life, during the RSV season(s), a RSV test was performed each time they experienced any symptoms of an ARTI. Infants were tested by a trained member of the study team at home or at the clinic and could be tested during more than 1 separate episode. The case-control study is a cross-sectional study performed in infants admitted to hospital, attending emergency departments (ED) or general practitioners (GPs) with symptoms of ARTI. Details of the study design and procedures can be found at clinicaltrials.gov (NCT03627572, NCT03756766). Informed consent was obtained from the parents of all study participants. All children with ARTI were eligible for RSV POC testing. For practical reasons, not all children could be tested with both the BN and the reference test. For this analysis, we included only samples on which both BN and a molecular reference test were performed (Figure 1).

Data on age, sex, comorbidities, duration of symptoms of ARTI, and level of medical care needed (hospitalized, medically attended [MA] ARTI, and non-MA ARTI) were obtained by completing questionnaires and case report forms. We defined 3 levels of medical care: (1) infants with ARTI who were hospitalized (including a subgroup of infants who were admitted to the pediatric intensive care unit [PICU]); (2) infants with MA ARTI, defined as infants who were seen at the ED or GP but were not admitted to the hospital; and (3) infants with non-MA ARTIs who did not see any doctor during the entire ARTI episode. In addition, the ReSViNET score was used to determine disease severity (Supplementary Table 1) [15].

Study procedures

A nasal flocked swab (FLOQSwab; Copan Diagnostics) was collected by a trained member of the study team and directly stored in one of the following viral transport media: MicroTest M4RT (3 mL; Remel) or UTM (3 mL; Copan Diagnostics). A maximum of 400 µL of the viral transport medium was used for POC testing. Samples were

transported at room temperature. The BN test was performed within 4 hours. The remaining sample was stored in aliquots at −80°C or discarded if RSV was negative (infant case-control study). The molecular reference test was either Xpert Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA) [16] or Alere i RSV assay (Alere Inc., Waltham, MA) [17] depending on availability of the tests at participating sites. The staff had hands-on training on how to sample patients and how to use the available POC tests before the start of the studies. All tests were performed according to the manufacturer's instruction. In short, for the BN assay, 100 µL of the viral transport medium mixed with the swab was aspirated with the included transfer pipette. The BN card was opened, and the entire content of the filled pipette was slowly expelled onto the sample pad of the device. A timer was set at 15 minutes to avoid inaccurate test results. After these 15 minutes, test results were read immediately from the BN test card, by visual inspection (Supplementary Text).

Statistical analysis

A positive molecular test for RSV was defined as the reference outcome. The BN results were compared with the reference test to measure diagnostic accuracy. Dichotomous variables were compared using $\chi 2$ or Fisher's exact test as appropriate. $P < .05$ were considered statistically significant. Univariate logistic regression analysis was used to determine whether false-negative BN tests results were associated with age, duration of symptoms, or ReSViNET score. Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY).

RESULTS

In total, 162 nasal swabs from 148 infants with symptoms of ARTI were tested with BN and the reference test. One hundred thirty-four infants were tested once and 14 infants were tested twice during 2 separate ARTI episodes. Of the 162 samples, 36 (22.2%) were from hospitalized infants, 83 (51.2%) from infants who had an MA ARTI, 41 (25.3%) from infants who had a non-MA ARTI, and 2 samples were from infants with missing data about level of care. Baseline characteristics are summarized in Table 1. Median age at moment of ARTI was 84 days (interquartile range, 39–178 days). Ninety-eight (78.4%) of the swabs were taken within 5 days after the start of symptoms. Four infants had comorbidities, including the following: prematurity, cardiomyopathy, and congenital bronchomalacia.

Table 1. Characteristics of Infants at Moment of ARTI Episode

Reference Test[a]		RSV Positive		RSV Negative
		BinaxNOW Positive	BinaxNOW Negative	BinaxNOW Negative
Total ARTI Episodes n = 162		(TP) n = 5	(FN) n = 61	(TN) n = 96
Age at moment of ARTI episode, days (median [IQR])	84 [39–178]	42 [33–203]	99 [49–197]	67 [34–161]
Sex, male (n, %)[b]	94 (58.0%)	4 (80.0%)	33 (54.1%)	57 (59.4%)
Comorbidity (n, %)[c]	4 (2.5%)	1 (20.0%)	3 (4.9%)	0 (0%)
Duration of symptoms[d] days (median [IQR])	3 [2–5]	4 [2–5]	3 [2–4]	3 [2–6]
Level of Care Needed (n, %)[e]				
Non-MA ARTI	41 (25.3%)	0 (0.0%)	5 (8.2%)	36 (37.5%)
MA ARTI	83 (51.2%)	3 (60.0%)	28 (45.9%)	52 (54.2%)
Hospitalized	36 (22.2%)	2 (40.0%)	28 (45.9%)	6 (6.3%)
PICU	11 (30.6%)	2 (100%)	7 (25.0%)	2 (33.3%)
Country (n, %)				
Netherlands	118 (72.8%)	3 (60.0%)	53 (86.9%)	62 (64.6%)
United Kingdom 14 (8.6%)		0 (0.0%)	0 (0.0%)	14 (14.9%)
Spain	30 (18.5%)	2 (40.0%)	8 (13.1%)	20 (20.8%)
ReSViNET score[f] 3 [1–6] (median [IQR])		6 [5–16]	5 [3–9]	1 [1–3]
Reference Test (n, %)				
Alere i RSV	120 (74.1%)	5 (100.0%)	32 (52.5%)	83 (86.5%)
Xpert Xpress	42 (25.9%)	0 (0.0%)	29 (47.5%)	13 (13.5%)
Flu/RSV				

Abbreviations: ARTI, acute respiratory tract infection; FN, false negative; IQR, interquartile range; MA ARTI, medically attended ARTI; n, number of ARTI episodes; PICU, pediatric intensive care unit; RSV, respiratory syncytial virus; TN, true negative; TP, true positive.
NOTE: Categorical data are expressed as frequency (%), and continuous data are expressed as median [IQR]. Percentages may not equal 100, because of rounding and missing values. P
values were not determined because of the low number of positive test results with BinaxNOW RSV.
[a]Alere i RSV or Xpert Xpress Flu/RSV were used as reference test.
[b]Including 10 males that were tested twice.
[c]None of the infants with comorbidity were tested twice.
[d]Data available for 125 episodes. [e]Data available for 160 episodes. [f]Data available for 99 episodes.

There were 66 RSV infections detected in 162 nasal swabs (40.7%), 5 (7.6%) of which tested positive by BN (Figure 1). All BN-positive samples also tested positive by the reference test. One infant had 2 RSV-positive episodes (1 episode of which was BN positive). Test characteristics of BN are shown in Table 2. Sensitivity was not significantly related to age, duration of symptoms, disease severity, or level of care required (Table 3). Sensitivity was higher in the subgroup of infants admitted to a PICU compared with other infants (22.2% versus 5.3%), although this difference was not statistically significant (P = .134). Univariate logistic regression analysis confirmed low sensitivity of BN in all subgroups.

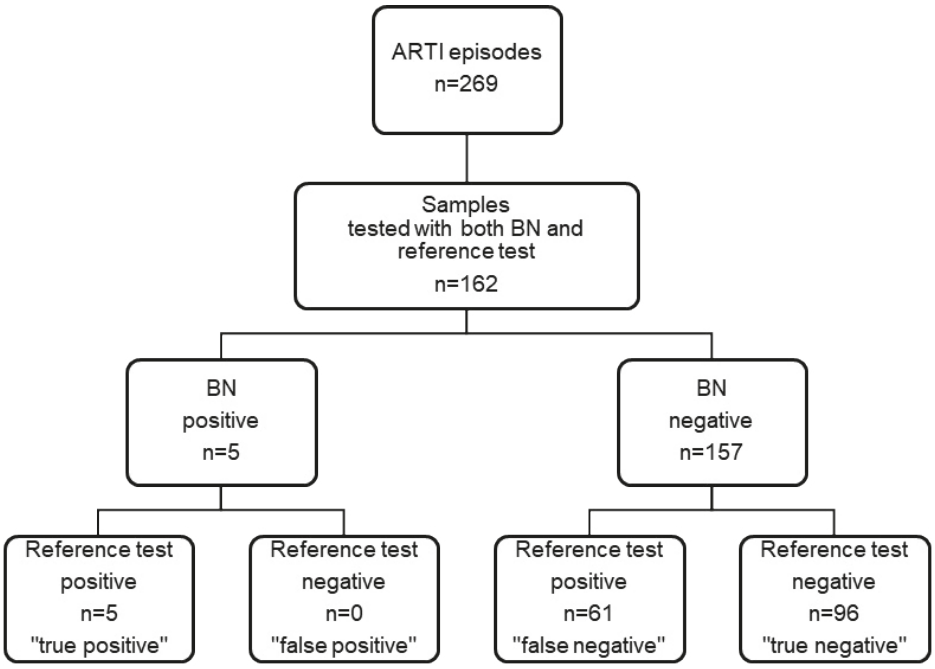

Figure 1: Study flow chart showing eligible acute respiratory tract infection (ARTI) episodes and test results of samples that were tested by BinaxNOW RSV (BN) and the reference test. n, number of ARTI episodes.
Abbreviations: ARTI, acute respiratory tract infection; n, number of ARTI episodes; BN, BinaxNOW® RSV; RSV, respiratory syncytial virus.

Table 2. Primary Analysis of BinaxNOW RSV Performance[a]

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Primary analysis (n=162)	7.6 (5/66)	100 (96/96)	100 (5/5)	61.1 (96/157)
95% CI (%)	*3.3-16.5*	*96.2-100.0*	*56.6-100.0*	*54.3-68.4*

Abbreviations: CI, confidence interval; n, number of acute respiratory tract infection episodes; NPV, negative predictive value; PPV, positive predictive value.
[a]Data are percentages (proportions) of BinaxNOW RSV test results compared with the reference test.

Table 3. BinaxNOW RSV Performance by Different Variables

Variable	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Level of Care Needed[a]				
Non-MA ARTI (n = 41)	0 (0/5)	100 (36/36)	NA (0/0)	87.8 (36/41)
MA ARTI (n = 83)	9.7 (3/31)	100 (52/52)	100 (3/3)	65.0 (52/80)
Hospitalized (n = 36)	6.7 (2/30)	100 (6/6)	100 (2/2)	17.6 (6/34)
PICU (n = 11)	22.2 (2/9)	100 (2/2)	100 (2/2)	22.2 (2/9)
P value	.726	NA	NA	<.005
Age				
≤60 days (n = 68)	12.5 (3/24)	100 (44/44)	100 (3/3)	67.7 (44/65)
>60 days (n = 93)	4.8 (2/42)	100 (51/51)	100 (2/2)	56.0 (51/91)
P value	.345	NA	NA	.183
Duration of Symptoms Before Testing[b]				
≤5 days (n = 98)	9.8 (5/51)	100 (47/47)	100 (5/5)	50.5 (47/93)
>5 days (n = 26)	0 (0/8)	100 (18/18)	NA (0/0)	69.2 (18/26)
P value	>.999	NA	NA	.119
ReSViNET score[c]				
≤3 (n = 53)	0 (0/17)	100 (36/36)	NA (0/0)	67.9 (36/53)
>3 (n = 46)	12.8 (5/39)	100 (7/7)	100 (5/5)	17.1 (7/41)
P value	.309	NA	NA	<.005

Abbreviations: ARTI, acute respiratory tract infection; MA ARTI, medically attended-ARTI; n, number of ARTI episodes; NA, not applicable; NPV, negative predictive value; PICU, pediatric intensive care unit; PPV, positive predictive value; RSV, respiratory syncytial virus.
NOTE: Data are percentages (proportions) of BinaxNOW RSV performance test results compared with the reference test. ReSViNET score was used to evaluate disease severity (Supplementary Figure 1).
[a]Data available for 160 episodes. bData available for 125 episodes. cData available for 99 episodes.

Table 4. Overview of Characteristics of Published Studies About the Performance of BN Compared With Molecular Tests in Children

Study	Age	Type of ARTI	Reference Test	Type of Sample	POC Setting	n=	Sensitivity (95% CI)	Specificity (95% CI)
Present study	<1 year (median 84 days)	Hospitalized, (non)-MA ARTI	Alere i, Xpert Xpress	NS (flocked swabs in 3 mL UTM/M4RT)	Yes	162	7.6% (3.3–16.5)	100% (96.2–100)
Bruning et al [18]	<16 years	Hospitalized (PICU), respiratory illness	RT-PCR	NPS, NPW	No	66	80% (64.3–95.7)	100% (NA)
Jung et al [19]	<2 years	Hospitalized, ALRI	RT-PCR	NPS in 1.5 mL VTM (in-house)	No	91	71.4% (61.4–79.7)	NA
Khanom et al [11]	<5 years	Hospitalized, ARTI	RT-PCR	NPS in 1 mL VTM (in-house)	Yes	159	41.2% (27.9–55.8)	100% (95.7–100)
Miernyk et al [7]	<3 years (mean 9.3 months)	Hospitalized, LRTI	RT-PCR	NPW	No	311	72% (61–74)	97% (94–99)
Mills et al [12]	<2 years (mean 7 months)	ED, respiratory symptoms	RT-PCR	NPA, NPW	Yes	579	83% (79–87)	83% (78–87)
Papenburg et al [20]	<3 years (median 5.7 months)	Hospitalized, ARTI	RT-PCR	NPA	No	720	80% (76–83.5)	96.9% (94–98.6)
Pfeil et al [21]	<3 years (mean 7.9 months)	Hospitalized, ARTI	RT-PCR	NW	Yes	242	63% (61–76)	100% (NA)

Abbreviations: ALRI, acute lower respiratory tract infection; ARTI, acute respiratory tract infection; BN, BinaxNOW RSV; CI, confidence interval; ED, emergency department; LRTI, lower respiratory tract infection; MA ARTI, medically attended ARTI; NA, not applicable; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; NS, nasal swab; NPW, nasopharyngeal wash; NW, nasal wash; PICU, pediatric intensive care unit; POC, point of care; RT-PCR, reverse-transcription polymerase chain reaction; VTM, viral transport media.

Test procedure

Because sensitivity of BN was lower than previously published, we carefully analyzed our procedures. Uniform standard operating procedures regarding sample collection and POC testing with BN was written and distributed to all participating centers before the start of the study. In the course of the study, BN test procedure was thoroughly evaluated, including a careful analysis by employees from the manufacturer (Supplementary Text). No technical explanation was found for the low sensitivity of BN.

DISCUSSION

In this study, we have shown that the overall sensitivity of BN was only 7.6% (95% CI, 3.3%–16.5%) in infants with ARTIs of varying clinical severity (hospitalized, MA ARTI, and non-MA ARTI). Highest sensitivity was seen in infants admitted to the PICU, although this was still only 22%. The sensitivity of BN in the current study is remarkably lower than previously reported. Two recent meta-analyses showed a pooled sensitivity of BN of 81% (95% CI, 74%–87%) [9] and 72.2% (95% CI, 65.2%–79.1%)

[10], respectively. Individual studies showed a sensitivity varying from 41.2% to 83% in children when compared with RT-PCR [7, 11, 12, 18–21]. Characteristics of these studies are shown in Table 4. The sample size of the studies varied between 66 and 720 participants with various age limitations. The 4 larger studies were all performed in children under the age of 3 years with nasopharyngeal aspirate (NPA) or nasal wash (NW) and showed a sensitivity of 63%–83% compared with RT-PCR. The 3 other studies were smaller and mainly used nasopharyngeal swab (NPS) as sampling method. The sensitivity of these studies varied between 41% and 80% compared with RT-PCR. The sample size of our study was 162, which is comparable but still smaller than the 4 larger studies. The low sensitivity in our study compared with the other studies is striking and necessitated a thorough analysis of the differences with the other studies and other possible explanations for the low sensitivity observed in our study. One of the differences between our study and the other studies is that we also included infants with non-MA ARTI, whereas other studies evaluated the performance of BN mainly in hospitalized children.

We reflected on possible explanations for the low sensitivity observed in our study. We considered that reduced disease severity could be linked to lower viral loads in infants recruited [22] and subsequently a lower sensitivity. However, even in the group of infants with severe disease who were admitted to hospital, sensitivity was less than 10%. Other factors that might influence sensitivity are age and duration of symptoms because both are probably related to viral load. False-negative results are more often seen with an increasing age [20] or longer duration of symptoms [7, 20, 21, 23]. However, all children in our study were younger than 1 year of age, and the majority (78.4%) were tested within 5 days after the start of symptoms, thus this could not explain the low sensitivity.

We also considered sampling methods as a cause of the low sensitivity in our study. Compared with the other published studies, we used nasal flocked swabs in 3 mL UTM or M4RT instead of NPS in 1 or 1.5 mL viral transport medium or NW/ NPA. We have previously shown that nasal aspirates are associated with higher sensitivity than nonflocked swabs to detect RSV by PCR [24]. Other studies have shown that sensitivity was comparable between NW or NPA and NPS with flocked swabs for detection of viruses by PCR [25, 26]. In addition, Blaschke et al [27] showed that midturbinate (nasal) flocked swabs are comparable to NPS for quantitative detection of RSV in infants, showing similar viral loads. Although no studies have previously compared the performance of rapid antigen testing in nasal swabs compared with aspirates or washes, we do not think that sampling methods fully explain the low sensitivity of BN. Temporal evolution of the binding site of the RSV fusion protein may have changed over time with loss of binding to the BN antibody, ultimately resulting in decreased sensitivity. We have limited information on viral sequences in our patient population. Because most of the known antigenic sites of the RSV fusion protein are generally well conserved, we believe this explanation for the low sensitivity of BN is unlikely [28]. Taken together, we have not found a methodological or biological explanation for the low sensitivity of BN in our study compared with previous reports.

A strength of our study is that it is part of a large prospective clinical study with a well defined study population performed in different centers across Europe. Our study is based on clinical endpoints rather than virological, ensuring a low risk of bias. Another strength is that we evaluated the performance in different clinical settings with a wide range of disease severity. This enabled us to evaluate test

performance not only in a hospital setting but also in primary care and EDs. Because the availability of POCTs is increasing, these tests might also be introduced into outpatient settings. Our study added valuable information about the sensitivity in different clinical settings, which is important to know before implementing POCTs in these settings. Finally, we evaluated the test procedure of BN thoroughly during the study period to avoid any bias due to incorrect handling of the tests (see Supplemental Text). We also worked closely with the manufacturer of BN to ensure we used the correct procedure.

There are several limitations to our study. First, we did not compare viral loads between true-positive and false-negative test results. Alere i and Xpert Xpress are qualitative tests. The RADT sensitivity depends on viral load [7, 8], whereas viral load is positively associated with disease severity [22]. In our study, sensitivity in the infants who were admitted to the PICU was higher, but this was still only 22% and not statistically significant higher compared with other clinical settings. Second, in our study, we used the Alere i RSV and Xpert Xpress Flu/RSV as reference standards, whereas RT-PCR has been used as the gold standard in some other studies [7, 11, 12, 18–20]. These new molecular assays are reported to have a sensitivity (93%–100%) and specificity (96%–100%) comparable with RT-PCR [29–34]. Third, we have not subtyped RSV. Respiratory syncytial virus genotype-B infection has been associated previously with false-negative results of RADT [20]. Fourth, we used nasal swabs and not NPS. Viral loads could be lower in this anterior nasal region and thus affect sensitivity. However, midturbinate flocked swabs have shown to be comparable for quantitative detection of RSV in infants [27]. Last, we have not analyzed why BN performed suboptimally. It is possible that both transport media used in this study, although recommended by the manufacturer, had some form of inhibitory effect on the test.

Conclusions

In conclusion, we have performed the first international prospective population-based study to define the sensitivity of a RADT for RSV infection. We showed that BN has low sensitivity in infants with ARTI in different clinical settings when collected with a nasal flocked swab in UTM or M4RT transport medium. Even in infants with the most severe disease, sensitivity was only 22%. Our study indicates that BN should be used and interpreted with caution. More studies are needed to determine variation in

sensitivity with different sampling methods. Physicians should consider using more sensitive molecular assays for RSV POC testing.

NOTES

Study group members

The RESCEU investigators are as follows: Roy Zuurbier; Louis Bont; Annefleur Langedijk; Mirjam Hamer; Koos Korsten; Marlies van Houten; Joanne Wildenbeest (University Medical

Center Utrecht); Simon Drysdale; Matthew Snape; Hannah Robinson; Andrew Pollard (University of Oxford);, Federico Martinón-Torres; Carmen Rodríguez-Tenreiro Sánchez; Alberto Gómez-Carballa; Ana Dacosta-Urbieta (Servicio Galego de Saude); Terho Heikkinen (Turku University Central Hospital); Steve Cunningham, Harish Nair, Harry Campbell, (University of Edinburgh); Peter Openshaw (Imperial College London); Philippe Beutels (Universiteit Antwerpen); Eva Molero (Synapse); Adam Meijer (National Institute for Public Health and the Environment); Thea Kølsen Fischer (Statens Serum Institut); Maarten van den Berge (Academisch Ziekenhuis Groningen); Carlo Giaquinto (PENTA Foundation); Mark Esser (AstraZeneca); Charles Knirsch (Pfizer); Amanda Leach (GlaxoSmithKline); Scott Gallichan, (Sanofi Pasteur); Jeroen Aerssens (Janssen); and Brian Rosen (Novavax).

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Conflict of interests

LJB has regular interaction with pharmaceutical and other industrial partners. He has not received personal fees or other personal benefits; he is also the founding chairman of the ReSViNET Foundation.

MDS, on behalf of the University of Oxford, has acted or acts as a Chief/Principal Investigator on research studies funded or sponsored by vaccine manufacturers including GlaxoSmithKline, Janssen, MCM, Novavax, Medimmune and Pfizer. He receives no personal financial benefit from this work.

AJP is Chair of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI) & the European Medicines Agency (EMA) scientific advisory group, on vaccines and is a member of the WHO's SAGE. AJP is an NIHR Senior Investigator. The views expressed in this article do not necessarily represent the views of DHSC, JCVI, NIHR or WHO.

FMT received honoraria from GSK, Pfizer, Sanofi Pasteur, MSD, and Janssen for taking part in advisory boards, expert meetings and for acting as speaker in congresses outside the scope of the submitted work. FMT has also acted as principal investigator in RCTs of the above-mentioned companies as well as Seqirus, Ablynx, Regeneron, Abbott, Novavax and Medimmune, with any honoraria being paid to his institution.

SC provides consultancy (including trial development and data monitoring) for which the University of Edinburgh receives payment from Janssen, Ablynx (Sanofi), Pulmocide, ReViral.

All remaining authors declare no competing interests.

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REFERENCES

1. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375:1545–55.
2. Stein RT, Bont LJ, Zar H, et al. Respiratory syncytial virus hospitalization and mortality: systematic review and meta-analysis. Pediatr Pulmonol 2017; 52:556–69.
3. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009; 360:588–98.
4. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017; 390:946–58.
5. van Houten CB, Naaktgeboren C, Buiteman BJM, et al. Antibiotic overuse in children with respiratory syncytial virus lower respiratory tract infection. Pediatr Infect Dis J 2018; 37:1077–81.
6. van Houten CB, Cohen A, Engelhard D, et al. Antibiotic misuse in respiratory tract infections in children and adults—a prospective, multicentre study (TAILORED Treatment). Eur J Clin Microbiol Infect Dis 2019; 38:505–14.
7. Miernyk K, Bulkow L, Debyle C, et al. Performance of a rapid antigen test (Binax NOW® RSV) for diagnosis of respiratory syncytial virus compared with real-time polymerase chain reaction in a pediatric population. J Clin Virol 2010; 50:240–3.
8. Prendergast C, Papenburg J. Rapid antigen-based testing for respiratory syncytial virus: moving diagnostics from bench to bedside? Future Microbiol 2013; 8:435–44.
9. Chartrand C, Tremblay N, Renaud C, Papenburg J. Diagnostic accuracy of rapid antigen detection tests for respiratory syncytial virus infection: systematic review and meta-analysis. J Clin Microbiol 2015; 53:3738–49.
10. Bruning AHL, Leeflang MMG, Vos JMBW, et al. Rapid tests for influenza, respiratory syncytial virus, and other respiratory viruses: a systematic review and meta-analysis. Clin Infect Dis 2017; 65:1026–32.
11. Khanom AB, Velvin C, Hawrami K, et al. Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. J Infect 2011; 62:52–8.
12. Mills JM, Harper J, Broomfield D, Templeton KE. Rapid testing for respiratory syncytial virus in a paediatric emergency department: benefits for infection control and bed management. J Hosp Infect 2011; 77:248–51.
13. Alere BinaxNOW RSV Card - Alere is now Abbott. Available at: https://www.alere.com/en/home/productdetails/binaxnow-rsv.html. Accessed 12 December 2018.
14. RESCEU. REspiratory Syncytial virus Consortium in EUrope. Available at: http://resc-eu.org/. Accessed 9 January 2019.
15. Justicia-Grande AJ, Pardo-Seco J, Cebey-López M, et al. Development and validation of a new clinical scale for infants with acute respiratory infection: the resvinet scale. PLoS One 2016; 11:1–15.
16. Cepheid Xpert Xpress Flu/RSV. Available at: http://www. cepheid.com/en/cepheid-solutions/clinical-ivd-tests/ critical-infectious-diseases/xpert-xpress-flu-rsv. Accessed 9 January 2019.
17. Alere i RSV - Alere is now Abbott. Available at: https:// www.alere.com/en/home/product-details/alere-i-rsv.html. Accessed 12 December 2018.

18. Bruning AHL, van Dijk K, van Eijk HWM, et al. Evaluation of a rapid antigen detection point-of-care test for respiratory syncytial virus and influenza in a pediatric hospitalized population in the Netherlands. Diagn Microbiol Infect Dis 2014; 80:292–3.
19. Jung BK, Choi SH, Lee JH, Lee JH, Lim CS. Performance evaluation of four rapid antigen tests for the detection of respiratory syncytial virus. J Med Virol 2016; 88:1720–4.
20. Papenburg J, Buckeridge DL, De Serres G, Boivin G. Host and viral factors affecting clinical performance of a rapid diagnostic test for respiratory syncytial virus in hospitalized children RADT rapid antigen detection test RSV respiratory syncytial virus RT-PCR reverse-transcriptase polymerase chain react. J Pediatr 2013; 163:911–3.
21. Pfeil J, Tabatabai J, Sander A, Ries M, Grulich-Henn J, Schnitzler P. Screening for respiratory syncytial virus and isolation strategies in children hospitalized with acute respiratory tract infection. Medicine (Baltimore) 2014; 93:e144.
22. Houben ML, Coenjaerts FEJ, Rossen JWA, et al. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. J Med Virol 2010; 82:1266–71.
23. Zhou L, Xiao Q, Zhao Y, Huang A, Ren L, Liu E. The impact of viral dynamics on the clinical severity of infants with respiratory syncytial virus bronchiolitis. J Med Virol 2015; 87:1276–84.
24. Meerhoff TJ, Houben ML, Coenjaerts FE, et al. Detection of multiple respiratory pathogens during primary respiratory infection: nasal swab versus nasopharyngeal aspirate using real-time polymerase chain reaction. Eur J Clin Microbiol Infect Dis 2010; 29:365–71.
25. Chan KH, Peiris JS, Lim W, Nicholls JM, Chiu SS. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. J Clin Virol 2008; 42:65–9.
26. Munywoki PK, Hamid F, Mutunga M, Welch S, Cane P, Nokes DJ. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real-time PCR using nasopharyngeal flocked swabs. J Clin Microbiol 2011; 49:3365–7.
27. Blaschke AJ, Mckevitt M, Ampofo K, et al. Midturbinate swabs are comparable to respiratory syncytial virus quantitative detection of nasopharyngeal swabs for in infants. J Pediatric Infect Dis Soc 2018; 1–5. doi:10.1093/jpids/ piy115/5194389.
28. Hause AM, Henke DM, Avadhanula V, Shaw CA, Tapia LI, Piedra PA. Sequence variability of the respiratory syncytial virus (RSV) fusion gene among contemporary and historical genotypes of RSV/A and RSV/B. PLoS One 2017; 12. doi:10.1371/journal.pone.0175792.
29. Azar MM, Landry ML. Detection of influenza a and b viruses and respiratory syncytial virus by use of clinical laboratory improvement amendments of 1988 (CLIA)-waived point-of-care assays: a paradigm shift to molecular tests. J Clin Microbiol 2018; 56. doi:10.1128/JCM.00367-18.
30. Peters RM, Schnee SV, Tabatabai J, Schnitzler P, Pfeil J. Evaluation of Alere i RSV for rapid detection of respiratory syncytial virus in children hospitalized with acute respiratory tract infection. J Clin Microbiol 2017; 55:1032–6.
31. Schnee SV, Pfeil J, Ihling CM, Tabatabai J, Schnitzler P. Performance of the Alere i RSV assay for point-of-care detection of respiratory syncytial virus in children. BMC Infect Dis 2017; 17:1–6.
32. Hassan F, Hays LM, Bonner A, et al. Multicenter clinical evaluation of the Alere i respiratory syncytial virus isothermal nucleic acid amplification assay. J Clin Microbiol 2018; 56:1–7.

33. Popowitch EB, Miller MB. A comparison of the Xpert Flu/RSV XC and Xpress Flu/RSV assays. J Clin Microbiol 2018; 56:1–3.
34. Banerjee D, Kanwar N, Hassan F, Essmyer C, Selvarangan R, Loeffelholz MJ. Comparison of six sample-to-answer influenza A/B and respiratory syncytial virus nucleic acid amplification assays using respiratory specimens from children. J Clin Microbiol 2018; 56:930–48.

SUPPLEMENTARY DATA

Supplementary text (test procedure BinaxNOW® RSV(BN))

Uniform standard operating procedures (SOP) regarding sample collection and POC testing with BN was written and distributed to all participating centers prior to the start of the study. The SOP contained a link to a video with detailed instructions how to use BN[13]. BN kits of different lot numbers were used. Positive and negative control swabs were tested before using tests from a new kit and were clearly positive or negative. All centers used microtipped flocked swabs to collect nasal mucus. The swab was immediately transferred into 3 ml virus transport medium (M4RT of UTM), both volume and type of virus transport medium (VTM) were acceptable according to the manufacturer's instruction. Swabs were rotated vigorously 3 times before breaking off the long end of the swab and leaving the swab in the medium. BN tests were performed within 4 hours after sample collection by slowly pipetting 100 µl from the medium on the card at the indicated point and sealing the card. Test results were read after 15 minutes, by visual inspection. Even a very faint sample line was interpreted as positive (according to the manufacturer's instructions). BN tests were performed by various researchers and research nurses at the different sites. BN false negative results were retested by another person for a subset of samples with the same results. Alere was contacted and checked the test procedure at location (UMCU), which they approved as being done according to the manufacturer's instruction. They were not able to explain the low observed sensitivity.

Supplementary Table 1.

	Item	0 points	1 points	2 points	3 points
1	**Feeding intolerance**	**No**	**Mild** Decreased appetite and/or isolated vomits with cough.	**Partial** Frequent vomits with cough, rejected feed but able to tolerate fluids sufficiently to ensure hydration.	**Total** Oral intolerance or absolute rejection of oral feed, not able to guarantee adequate hydration orally. Required nasogastric and/or intravenous fluids
2	**Medical intervention**	**No**	**Basic** Nasal secretions aspiration, physical examination, trial of nebulized bronchodilators, antipyretics.	**Intermediate** Oxygen therapy required. Complementary exams were needed (chest X-rays, blood gases, hematimetry…). Maintained nebulized therapy with bronchodilators.	**High** Required respiratory support with positive pressure (either non-invasive in CPAP, BiPAP or high-flow O2; or invasive through endotracheal tube).
3	**Respiratory difficulty**	**No**	**Mild** Not in basal situation but does not appear severe. Wheezing only audible with stethoscope, good air entrance. If modified Wood Downes, Wang score or any other respiratory distress score is applied, it indicates mild severity.	**Moderate** Makes some extra respiratory effort (intercostal and/or tracheosternal retraction). Presented expiratory wheezing audible even without stethoscope, and air entrance may be decreased in localized areas. If modified Wood Downes, Wang score or any other respiratory distress score is applied, it indicates moderate severity.	**Severe** Respiratory effort is obvious. Inspiratory and expiratory wheezing and/or clearly decreased air entry. If modified Wood Downes, Wang score or any other respiratory distress score is applied, it indicates high severity.
4	**Respiratory frequency**	**Normal** < 2 m: 40–50 bpm 2–6 m: 35–45 bpm 6-12m: 30–40 bpm 12-24m:25–35 bpm 24-36m: 20–30 bpm	**Mild or occasional tachypnea** Presented episodes of tachypnea, well tolerated, limited in time by self-resolution or response to secretion aspiration or nebulization.	**Prolonged or recurrent tachypnea** Tachypnea persisted or recurred despite secretion aspiration and/or nebulization with bronchodilators.	**Severe alteration** Severe and sustained tachypnea. Very superficial and quick breath rate. Normal/low breath rate with obvious increased respiratory effort and/or mental status affected. Orientative rates of severe tachypnea: < 2 m: > 70 bpm 2–6 m: > 60 bpm 6-12m: >55 bpm 12-24m: >50 bpm 24-36m: >40 bpm
5	**Apnea**	**No**			**Yes** At least one episode of respiratory pause medically documented or strongly suggested through anamnesis.
6	**General Condition**	**Normal**	**Mild** Not in basal situation, child was mildly uncomfortable but does not appear to be in a severe condition, not impress of severity. Parents are not alarmed. Could wait in the waiting room or even stay at home.	**Moderate** Patient looks ill, and will need medical exam and eventually further complementary exams and/or therapy. Parents are concerned. Cannot wait in the waiting room.	**Severe** Agitated, apathetic, lethargic. No need of medical training to realize severity. Parents are very concerned. Immediate medical evaluation and/or intervention were required.
7	**Fever**	**No**	**Yes, mild** Central T < 38.5°C	**Yes, moderate** Central T > 38.5°C	

(m = months)

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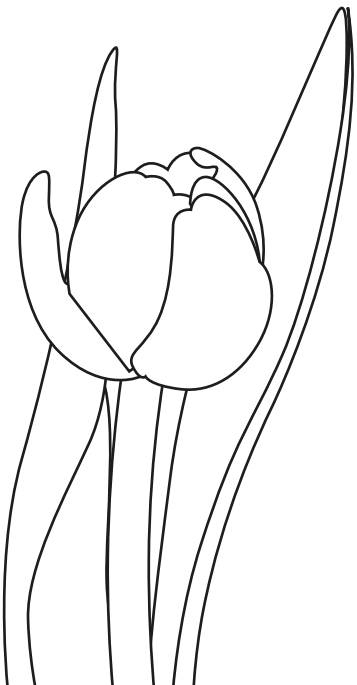
ReSViNET score: Each clinical symptom is scored according to the description provided. The total score forms the ReSViNET score (0-20).[1]

1. Justicia-Grande AJ, Pardo-Seco J, Cebey-López M, et al. Development and validation of a new clinical scale for infants with acute respiratory infection: The resvinet scale. PLoS One. **2016**; 11(6):1–15.

It's easier to stick to the old
(The War On Drugs – Red Eyes)



Chapter 3

Performance Assessment of a Rapid Molecular Respiratory Syncytial Virus Point-of-Care Test: A Prospective Community Study in Older Adults

Roy P. Zuurbier, Koos Korsten, Theo J.M. Verheij, Chris Butler, Niels Adriaenssens, Samuel Coenen, Olivier Gruselle, Valerie Vantomme, Marlies A. van Houten, Louis J. Bont, Joanne G. Wildenbeest, for the RESCEU Investigators

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ABSTRACT

Background. Respiratory syncytial virus (RSV) causes a substantial burden in older adults. Viral load in RSV-infected adults is generally lower compared to young children which could result in suboptimal sensitivity of RSV diagnostics. Although the Xpert® Xpress Flu/RSV assay has been used in routine clinical care, its sensitivity to diagnose RSV infection in older adults is largely unknown. We aimed to compare the performance of the Xpert® Xpress Flu/RSV assay with real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in home-dwelling older adults (≥60 years).

Methods. Nasopharyngeal swabs were tested with Xpert® Xpress Flu/RSV and compared to RSV RT-PCR in older adults with acute respiratory tract infections (ARTIs) with different levels of disease severity.

Results. We studied 758 respiratory samples from 561 older adults from two consecutive RSV seasons. Thirty-five (4.6%) samples tested positive for RSV by at least one of the assays, of which two samples were negative by Xpert® Xpress Flu/RSV and three samples by real-time RT-PCR. The positive percentage agreement (PPA) was 90.9% (95% Confidence Interval (CI) 76.4-96.8%) and negative percentage agreement (NPA) was 99.7% (95% CI 99.0-99.9%). Viral loads were low (≤10^3 copies/mL or Ct-value ≥34) in all cases with discordant results for the two assays.

Conclusion. The PPA of Xpert® Xpress Flu/RSV compared to routine RT-PCR is high for RSV detection in home-dwelling older adults. The assay is fast and easy to use at the point of care.

Clinical Trials Registration. NCT03621930

Keywords

Respiratory syncytial virus, diagnosis, molecular, point-of-care test, older adults

BACKGROUND

Lower respiratory tract infections (LRTIs) are estimated to be the fifth-leading cause of mortality worldwide [1]. Respiratory syncytial virus (RSV) is a major cause of respiratory infections in older adults (≥60 years) with a substantial disease burden [2–5]. The annual incidence rate of RSV infection in community-dwelling older adults is estimated at 3% to 7.2% [6,7]. It was estimated that approximately 14,000 (range: 5000 to 50,000) in-hospital deaths due to acute respiratory infections in older adults were related to RSV in 2015 [3].

Currently, the gold standard for RSV diagnosis is laboratory-based real-time reverse transcriptase polymerase chain reaction (RT-PCR). RT-PCR has the disadvantage that it requires technical skills and has a long turnaround time. Therefore, reliable rapid diagnostic tests are needed to improve patient management, to enable cohorting and isolation of hospitalised patients, to prevent unnecessary use of antibiotics [8], and for the use of RSV antivirals for treatment in the near future [9].

In recent years, several point-of-care tests (POCTs) have been developed to detect RSV amongst other rapid antigen diagnostic tests (RADTs) and molecular assays. In general RADTs are less sensitive compared to molecular POCTs. PCR-based molecular POCT assays are available and used in clinical practice because they are fast, easy to use by non-laboratory personnel, and could be less expensive compared to routine RT-PCR, however they are less suitable for high throughput. The turnaround time of most molecular POCTs is less than one hour. The use of molecular POCTs is associated with a significant reduction in hospital length of stay, testing costs, and isolation time [10,11]. The Xpert® Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA) is one of the commercially available molecular POCTs [12]. It is a real-time RT-PCR assay using a single disposable cartridge. Previous studies reported a sensitivity and specificity for RSV ranging from 90.5% to 100% and 99.6% to 100%, respectively [13–17]. However, these studies bear the risk of overestimating test accuracy as they were performed in medically attended or hospitalized patients [13–16], used remnant specimens [13,15], were partially performed in children with predictable high viral loads [14,15], were mostly sponsored by the manufacturer [14–16], and were performed in relatively small numbers of patients [13,15,17]. In an earlier report, we showed an unexpectedly low sensitivity for RADT BinaxNOW® RSV in infants with

different levels of care, thereby demonstrating the importance of validating these POCTs in different populations [18].

The aim of the current study was to evaluate the performance of the Xpert® Xpress Flu/RSV assay [19] to diagnose RSV infection in home-dwelling older adults (≥60 years) with acute respiratory tract infection (ARTI) in different clinical settings as part of a large international prospective cohort study.

METHODS

Study population

The study population consisted of older adults (≥60 years of age) with an acute respiratory tract infection (ARTI) who were participating in the REspiratory Syncytial virus Consortium in EUrope (RESCEU) [7] older adult cohort study during two consecutive RSV seasons, 2017-2018 and 2018-2019. RESCEU is an EU-funded consortium aiming to determine RSV burden of disease in Europe. The study was performed in Belgium (Antwerp), the Netherlands (Utrecht), and the United Kingdom (Oxford). Participants were recruited from 17 general practices before the start of each RSV season. A total of 1,040 community-dwelling older adults participated in the study of whom approximately 50% were above 75 years of age. Participants were followed during one RSV season, between the 1st of October and 30th of April, nasopharyngeal swabs were collected for RSV testing each time a participant experienced an ARTI. Participants were contacted weekly by email or telephone during the RSV-season to ask for symptoms of ARTI, which was defined as the presence of one or more of the following symptoms for at least one day: cough, nasal congestion or discharge, wheezing, or shortness of breath. Samples were taken by a trained member of the study team at home. Details of the study design and procedures have been previously described [7].

Older adults were defined as adults of age 60 or older. Data on age, sex, comorbidities, duration of symptoms of ARTI, and level of medical care needed were obtained by completing questionnaires and case report forms (CRFs). We defined three levels of medical care: (i) participants with ARTI who were hospitalized, (ii) participants with medically attended (MA) ARTI, defined as participants who were seen at the emergency department (ED) or general practice but were not admitted to the

hospital, and (iii) participants with non-MA ARTI who did not see any clinician during the entire ARTI episode. Informed consent was obtained from all study participants.

Study procedures

Two nasopharyngeal minitip flocked swabs (FLOQSwab™, Copan diagnostics) were collected by a member of the study team and directly stored in UTM™ (Copan diagnostics, 3 mL) and MicroTest™ M4RT® (Remel, 3 mL), respectively. Samples were transported at room temperature. 300 µL of UTM™ was used for POC analysis by the Xpert® Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA, USA)[19]. POC testing was performed within 24 hours. The remaining UTM sample was discarded. The MicroTest™ M4RT® sample was stored in aliquots at −80 °C for later analysis by RT-PCR assay. The staff was trained on how to sample patients and how to use the POCT before the start of the study.

Virology

Both assays reported information on viral load (Cycle threshold (Ct) value for Xpert® Xpress and copies/mL for RT-PCR). The Xpert® Xpress POCT was performed according to the manufacturer's instruction. In short, 300 µL of the viral transport medium mixed with the swab was aspirated with the included transfer pipette. The cartridge was opened and the entire content of the filled pipette was slowly expelled into the cartridge. Subsequently, the cartridge was inserted into the GeneXpert System. After approximately 30 minutes test results were available on the screen. The assay targeted the RSV N gene, encoding the RSV nucleocapsid, using three RSV A and two RSV B strains [20]. A test was positive if the threshold was reached before completion of the full 40 PCR cycles. In case of a positive test, RSV viral load was reported as a Ct value.

For RT-PCR an in-house developed kit was used. RSV A and B were detected and quantified by duplex RT-PCR using specific amplification primers and fluorescent probes designed to detect the RSV N gene. The process involves extraction of nucleic acids, conversion of RNA to complementary deoxyribonucleic (DNA) by reverse transcription, and detection by real-time PCR reaction using a calibration curve (absolute quantitation). 200 µl of M4RT from nasal swab samples were used for the nucleic acid extraction (KingFisher, MagMax Core kit). Nucleic acids were eluted in a volume of 80 µl, 2.5 µl of the elution was used per RT-PCR amplification. Limit of detections (LODs) were determined via probit approach, as recommended in the CLSI

EP17-A2 guidance. Several dilutions of surrogate samples (M4RT transport medium spiked with different concentrations of RSV-A and RSV-B strains) were used for their determinations. The RSV A RT-PCR has a LOD of 304 copies/mL, while the LOD for the RSV B RT-PCR is 475 copies/mL. Clinical samples were considered positive when the load was higher than the respective LODs. RT-PCR of all samples was done at the same moment and location.

Statistical analysis

Only samples tested with both assays were included in the analysis. Test results of the Xpert® Xpress assay were compared to routine real-time RT-PCR as reference standard using positive percentage agreement (PPA), negative percentage agreement (NPA) and overall rate of agreement (ORA). Using percentage agreement rather than accuracy and sensitivity is recommended by the FDA when comparing results of a new test with an imperfect reference test, as RT-PCR is not 100% accurate and comparable to molecular POCT tests [21]. Confidence intervals were calculated using the Wilson score test. Patient characteristics were compared between the four outcome categories using chi-square or Fisher's exact test for categorical data and Mann-Whitney-U test for continuous data. P values <0.05 were considered statistically significant. Multivariate logistic regression analysis was used to determine whether PPA of the tests was associated with age, duration of symptoms, or level of care. In these models PPA was used as binary outcome defined as results positive for both assays and positive RT-PCR results combined with negative POCT result. Statistical analyses were conducted using R version 3.6.1 within RStudio version 1.2.5.

RESULTS

Acute respiratory tract infections

In total, 758 samples from 561 participants with symptoms of ARTI were tested with Xpert® Xpress Flu/RSV and RT-PCR (Figure 1). Eighty-six ARTI episodes were excluded because one or both tests were not performed. Characteristics of excluded episodes did not differ from the included episodes except for country and severity, showing that significantly more hospitalizations and MA ARTI episodes did not have both tests performed (Supplementary Table 1). The median age of participants at the time of ARTI was 75 years (IQR 67 to 80 years). Comorbidity was present in 291 (38.4%) participants, including cardiac disease, pulmonary disease, and diabetes (Supplementary Table 2). 396 participants were tested once, 136 twice, and 29 were

tested three times or more (maximum 4 times) during separate ARTI episodes. Sample collection and participant characteristics of the four outcome categories are displayed in Table 1 and showed no significant differences between categories. Swabs were taken after a median duration of symptoms of 4 days (IQR 2 to 6 days). Most respiratory episodes were mild with only 4 (0.5%) hospitalizations and 170 (22.4%) MA ARTI episodes (Table 1).

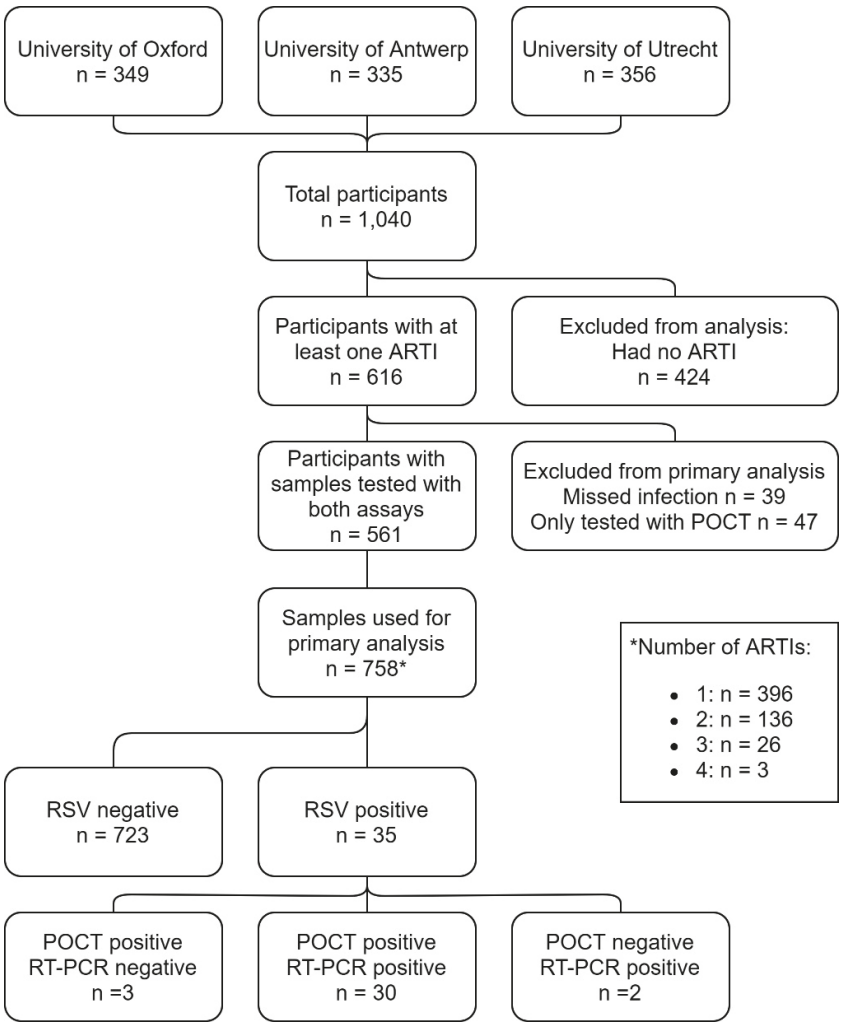

Figure 1. Flowchart of participants of the REspiratory Syncytial Virus Consortium in EUrope (RESCEU) older adult cohort study with at least 1 acute respiratory tract infection (ARTI) during follow-up. Respiratory syncytial virus (RSV)–negative cases were negative by both reverse-transcription polymerase chain reaction (RT-PCR) and Xpert® Xpress Flu/RSV (point-of-care test [POCT]).

Table 1. Sample Characteristics, Stratified by Test Assay Result

Characteristic	POCT⁻/RT-PCR⁻	POCT⁺/RT-PCR⁺	POCT⁻/RT-PCR⁺	POCT⁺/RT-PCR⁻
No. of episodes	723	30	2	3
Country				
Belgium	222 (30.7)	11 (36.7)	0 (0.0)	1 (33.3)
Netherlands	283 (39.1)	13 (43.3)	2 (100.0)	0 (0.0)
United Kingdom	218 (30.2)	6 (20.0)	0 (0.0)	2 (66.7)
Duration of symptoms at moment of sample collection, d, median (IQR)	4 (2–6)	3 (2–5)	4.5 (3–6)	6 (4.5–6)
Sex, female	391 (54.1)	15 (50.0)	2 (100.0)	2 (66.7)
Comorbidity	279 (38.6)	9 (30.0)	2 (100.0)	1 (33.3)
Age at ARTI episode, y, median (IQR)	75 (67–80)	75 (70–79.5)	69 (66.5–71.5)	79 (78.5–79)
Level of care needed				
Hospitalized	4 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
MA ARTI	160 (22.1)	9 (30.0)	0 (0.0)	1 (33.3)
Non–MA ARTI	551 (76.2)	21 (70.0)	2 (100.0)	2 (66.7)
Not known	8 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)

Data are presented as No. (%) unless otherwise indicated. Categories are based on Xpert® Xpress Flu/RSV (POCT) and RT-PCR test results.
Abbreviations: –, negative; +, positive; ARTI, acute respiratory tract infection; IQR, interquartile range; MA, medically attended; POCT, point-of-care test; RT-PCR, reverse-transcription polymerase chain reaction.

Table 2. Primary Outcome: Performance of Cephei d Xpert® Xpress Flu/RSV Compared With Reverse-Transcription Polymera ase Chain Reaction

Test	PPA, %	NPA, %	ORA, %
Xpert® Xpress Flu/RSV (n = 758 ARTI episodes)	90.9 (30/33)	99.7 (723/725)	99.3 (753/758)
95% CI, %	76.4–96.8	99.0–99.9	98.5–99.7

Abbreviations: ARTI, acute respiratory tract infection; CI, confidence interval; NPA, negative percentage agreement; ORA, overall rate of agreement; PPA, positive percentage agreement.

Table 3. Characteristics of Patients With Discordant Test Results Between Xpert® Xpress Flu/RSV (Point-of-Care Test) and Reverse-Transcription Polymerase Chain Reaction

Test Result	RSV Subtype	Ct Value POCT	RT-PCR, Viral Copies/mL (log_{10})	Duration of Symptoms at Moment of Sample Collection, d	Level of Care	Age, y	Sex
POCT^{+}/ RT-PCR^{-}	RSV-B	36.0	399[a] (2.6)	3	Non-MA ARTI	78	Female
POCT^{+}/ RT-PCR^{-}	RSV-B	34.8	290[a] (2.5)	6	MA ARTI	79	Female
POCT^{+}/ RT-PCR^{-}	NA	36.1	0	6	Non-MA ARTI	79	Male
POCT^{-}/ RT-PCR^{+}	RSV-A	NA	532 (2.7)	7	Non-MA ARTI	64	Female
POCT^{-}/ RT-PCR^{+}	RSV-B	NA	1080 (3.0)	2	Non-MA ARTI	74	Female

RSV genotype was determined by RT-PCR.

Abbreviations: –, negative; +, positive; ARTI, acute respiratory tract infection; Ct, cycle threshold; MA, medically attended; NA, not available; POCT, point-of-care test; RSV, respiratory syncytial virus; RT-PCR, reverse-transcription polymerase chain reaction.

[a]Below limit of detection for RSV-B (475 copies/mL).

RSV-ARTI

RSV was detected in 35 samples (4.6%) by at least one of the assays (33 by Xpert® Xpress, and 32 by RT-PCR). We found a PPA of 90.9% (95% CI, 76.4-96.8), and a NPA of 99.7% (95% CI, 99.0-99.9) for Xpert® Xpress Flu/RSV compared to RT-PCR (Table 2). The ORA between both tests was 99.3% (95% CI 98,5-99.7). Five samples showed discordant test results (Table 3). All discordant samples had a low viral load (≤10^3 copies/mL or Ct-value ≥34). Two out of the three samples tested positive by Xpert® Xpress and negative by RT-PCR, showed a low number of RSV viral copies with RT-PCR, but did not meet the threshold of viral copies to be considered positive. We found a moderately strong correlation between the Xpert® Xpress Flu/RSV and RT-PCR for viral load (Pearson's r = -0.70, p<0.001, Figure 2). We found no significant effects of age, gender, duration of symptoms, comorbidity, and level of care on PPA using multivariate logistic regression tests.

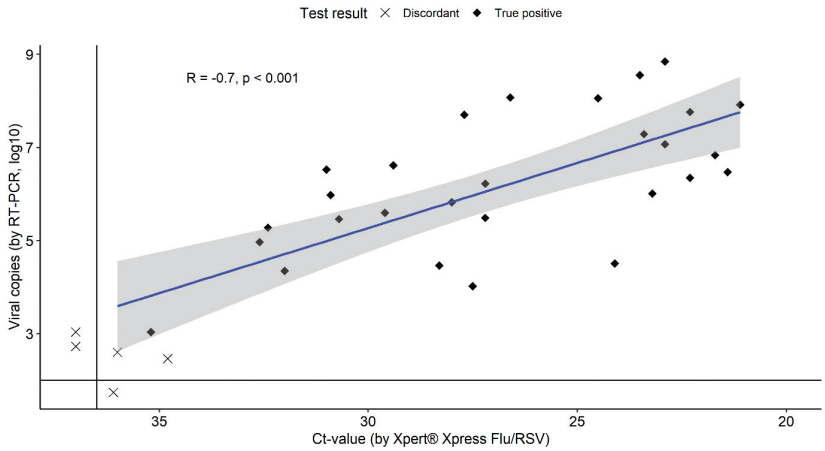

Figure 2. Scatterplot of viral copies/mL (log) by reverse-transcription polymerase chain reaction (RT-PCR) and cycle threshold (Ct) value by Xpert® Xpress Flu/RSV (point-of-care test). Black diamond dots are tested positive with both assays. Crosses indicate discordant test results. Dots below the horizontal or left of the vertical lines are undetectable by 1 of the assays. The blue line shows the regression line with confidence interval (gray shadow).

DISCUSSION

This is the first community-based study of the performance of Xpert® Xpress Flu/RSV in older adults. We found a high PPA and ORA between Xpert® Xpress and RT-PCR (90.9% and 99.3%, respectively) for RSV detection. Test failure for either test was exclusively observed in patients with low viral load, around or below LoD of both tests.

Results of our study are comparable with previous studies which were performed in a clinical setting or in hospitalized patients showing a sensitivity of 90.5% to 100% [13–17]. The sample size of these studies varied between 172 and 2,553 participants with a mixed age spectrum (infants to older adults), with RSV positivity varying from 3.5% to 55%. All studies used a nasopharyngeal swab or nasopharyngeal aspirate as the sampling method and used RT-PCR as the reference. The low viral load in all cases of discordant test results in our study was in line with earlier reports [16].

A strength of our study is that it is part of a large prospective clinical study with a well-defined, community-based population and performed in different countries across Europe. Our study design was based on clinical endpoints rather than virological, ensuring a low risk of bias. Therefore, we were able to evaluate the

performance of Xpert® Xpress Flu/RSV in a community setting that included mild disease, while other studies only evaluated the performance of Xpert® Xpress Flu/RSV in a clinical setting or in hospitalized patients. Second, rather than using sensitivity to present concordance between both tests, we used PPA to describe the performance of Xpert® Xpress Flu/RSV compared to RT-PCR. While real-time RT-PCR is widely used as the gold standard for virus detection, there is no assay with 100% accuracy. Most molecular POCT assays are using the same nucleic amplification method as RT-PCR and are known for their high sensitivity and specificity, similar to RT-PCR [12]. This way of displaying results is recommended when comparing results of a new test with an imperfect reference test [21].

There are several limitations to our study. First, we used UTM viral transport medium for analysis with Xpert® Xpress according to the manufacturer's instruction, and M4RT for RT-PCR analysis, both with different nasopharyngeal swabs and analyzed at different time points. This could have had an effect on viral load of the specimens. Both nasopharyngeal swabs were taken at the same moment by the same research personnel to minimize any possible effects on viral load. However, with low viral loads this could lead to a difference in test results. To our knowledge there is no literature on viral transport media affecting viral load. For both tests we used the recommended viral transport medium. The M4RT samples were stored at −80 °C until testing. This temperature allows long-term sample storage without significant effects on quality of samples. Second, as this is a community-based cohort study, the number of RSV-positive samples was relatively low (n=35; 4.6%). However, we are confident that our results are reliable, based on the high concordance between both tests in a representative range of viral loads. Third, 10.2% of ARTI episodes could not be used for this study because none or only one assay was performed. Although significantly more hospitalizations and MA ARTI episodes were missed, we do not believe this had an impact on our results because other studies have previously shown a high sensitivity in these populations [13–17]. Last, although Xpert® Xpress Flu/RSV also reported influenza results of tested samples, influenza virus was not tested by RT-PCR for practical reasons. Influenza results of Xpert® Xpress Flu/RSV in our cohort have been described previously [7].

RSV is a significant cause of moderate-to-severe respiratory tract infection in older adults [22]. Early detection of the virus can improve patient management and outcomes [23]. In addition, rapid testing can be important as companion diagnostics

for use of future RSV antivirals at an early stage [24]. Molecular POC assays are highly sensitive and easy to use. The Xpert® Xpress Flu/RSV assay is among the four low-complex Clinical Laboratory Improvement Amendments (CLIA)-waived molecular assays, approved by the US Food and Drug Administration (FDA) [12]. It is performed on the Cepheid GeneXpert® System, which can also be used for multiple other pathogens and is suitable for testing up to 16 samples at the same time. Hands-on time is estimated to be 1 to 2 minutes, and turnaround time is about 30 minutes [12,13]. As the availability of molecular POCTs is increasing, these assays might also be introduced into outpatient settings. Our study added valuable information about the PPA in patients who needed different levels of care to existing literature, which is important to know before implementing molecular POCTs in these settings.

In conclusion, we have performed the first international prospective community-based study to compare the performance of a rapid molecular detection test for RSV infection with RT-PCR in home-dwelling older adults. We demonstrated that the PPA and ORA between Xpert® Xpress Flu/RSV and routine RSV RT-PCR for RSV detection in home-dwelling older adults is high. The assay is fast and easy to use and therefore has the ability to improve patient management and outcomes.

NOTES

Study group members

The RESCEU investigators are as follows: Roy Zuurbier, Koos Korsten, Theo Verheij, Marlies van Houten, Louis Bont, Joanne Wildenbeest (University Medical Centre Utrecht), Niels Adriaenssens, Samuel Coenen (University of Antwerp), Christopher Butler, Andrew Pollard (University of Oxford), Jean-Yves Pirçon, Olivier Gruselle, Amanda Leach (GlaxoSmithKline); Harish Nair, Harry Campbell (University of Edinburgh); Philippe Beutels (University of Antwerp); Peter Openshaw, (Imperial College London); Federico Martinon-Torres (Servicio Galego de Saude), Terho Heikkinen (University of Turku and Turku University Hospital), Adam Meijer (National Institute for Public Health and the Environment); Thea Kølsen Fischer (Statens Serum Institut); Maarten van den Berge (Academisch Ziekenhuis Groningen); Carlo Giaquinto (Fondazione PENTA for the Treatment and Care of Children with HIV-ONLUS); Michael Abram (AstraZeneca); Kena Swanson (Pfizer); Jeroen Aerssens (Janssen); Clarisse Demont, Scott Gallichan (Sanofi Pasteur); Brian Rosen (Novavax); Eva Molero (Team-It Research).

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Conflict of interests
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Ethical approval
This study was approved by the Ethical Review Authority in Belgium (reference No B300201732907), The Netherlands (reference No NL60910.041.17), and United Kingdom (Ethics ref 17/LO/1210, IRAS Ref: 224156). Participants gave informed consent before taking part in this study. The study was conducted according with the Declaration of Helsinki, as revised in 2013.

REFERENCES

1. Troeger C, Forouzanfar M, Rao PC, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis 2017; 17:1133–61.
2. Shi T, Arnott A, Semogas I, et al. The etiological role of common respiratory viruses in acute respiratory infections in older adults: a systematic review and meta-analysis. J Infect Dis 2020; 222(Suppl 7):S563–9.
3. Shi T, Denouel A, Tietjen AK, et al. Global disease burden estimates of respiratory syncytial virus–associated acute respiratory infection in older adults in 2015: a systematic review and meta-analysis. J Infect Dis 2020; 222(Suppl 7):S577–3.
4. Fleming DM, Taylor RJ, Lustig RL, et al. Modelling estimates of the burden of respiratory syncytial virus infection in adults and the elderly in the United Kingdom. BMC Infect Dis 2015; 15:443.
5. Bruyndonckx R, Coenen S, Butler C, et al. Respiratory syncytial virus and influenza virus infection in adult primary care patients: association of age with prevalence, diagnostic features and illness course. Int J Infect Dis 2020; 95:384–90.
6. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005; 352:1749–59.
7. Korsten K, Adriaenssens N, Coenen S, et al. Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study. Eur Respir J 2020; 352:2002688.
8. Houten CB van Cohen A, Engelhard D, et al. Antibiotic misuse in respiratory tract infections in children and adults—a prospective, multicentre study (TAILORED Treatment). Eur J Clin Microbiol Infect Dis 2019; 38:505–14.
9. Mazur NI, Martinón-Torres F, Baraldi E, et al. Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. Lancet Respir Med 2015; 3:888–900.
10. Wabe N, Li L, Lindeman R, et al. Impact of rapid molecular diagnostic testing of respiratory viruses on outcomes of adults hospitalized with respiratory illness: a multicenter quasi-experimental study. J Clin Microbiol 2019; 57:e01727-18.
11. Rogers BB, Shankar P, Jerris RC, et al. Impact of a rapid respiratory panel test on patient outcomes. Arch Pathol Lab Med 2015; 139:636–41.
12. Hogan AC, Caya C, Papenburg J. Rapid and simple molecular tests for the detection of respiratory syncytial virus: a review. Expert Rev Mol Diagn 2018; 18:617–29.
13. Ling L, Kaplan SE, Lopez JC, Stiles J, Lu X, Tang Y-W. Parallel validation of three molecular devices for simultaneous detection and identification of influenza a and b and respiratory syncytial viruses. J Clin Microbiol 2017; 56:e01691-17.
14. Cohen DM, Kline J, May LS, et al. Accurate PCR detection of influenza A/B and respiratory syncytial viruses by use of Cepheid Xpert Flu+RSV Xpress assay in point-of-care settings: comparison to Prodesse ProFlu. J Clin Microbiol 2017; 56:e01237-17.
15. Popowitch EB, Miller MB. Comparison of the Xpert Flu/ RSV XC and Xpress Flu/RSV assays. J Clin Microbiol 2018; 56:e00278-18.
16. Zou X, Chang K, Wang Y, et al. Comparison of the Cepheid Xpert Xpress Flu/RSV assay and commercial real-time PCR for the detection of influenza A and influenza B in a prospective cohort from China. Int J Infect Dis 2019; 80:92–7.

17. Ho YII, Wong AH, Lai RWM. Comparison of the Cepheid Xpert Xpress Flu/RSV assay to in-house Flu/RSV triplex real-time RT-PCR for rapid molecular detection of influenza A, influenza B and respiratory syncytial virus in respiratory specimens. J Med Microbiol 2018; 67:1576–80.
18. Zuurbier RP, Bont LJ, Langedijk AC, et al. Low sensitivity of BinaxNOW RSV in infants. J Infect Dis 2020; 222(Suppl 7):S640–47.
19. Cepheid. Xpert Xpress Flu/RSV. http://www.cepheid.com/en/cepheid-solutions/clinical-ivd-tests/critical-infectious-diseases/xpert-xpress-flu-rsv. Accessed 9 January 2019.
20. Cepheid. Product insert: Xpert Xpress Flu/RSV. CE-IVD 301-7239, Rev C. 2019. https://www.cepheid.com/PackageInsertFiles/Xpress-Flu-RSV-US-IVD-ENGLISH-Package-Insert-301-7239-Rev. C.pdf. Accessed 28 April 2020.
21. Food and Drug Administration. Guidance for industry and FDA staff statistical guidance on reporting results from studies evaluating diagnostic tests. 2007. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.htm. Accessed 27January 2021.
22. Kestler M, Muñoz P, Mateos M, Adrados D, Bouza E. Respiratory syncytial virus burden among adults during flu season: an underestimated pathology. J Hosp Infect 2018; 100:463–8.
23. Lee N, Walsh EE, Sander I, et al. Delayed diagnosis of respiratory syncytial virus infections in hospitalized adults: individual patient data, record review analysis and physician survey in the United States. J Infect Dis 2019; 220:969–79.
24. Löwensteyn YN, Bont LJ. Clinical development of respiratory syncytial virus antivirals—what we can learn from oseltamivir. Clin Infect Dis 2020; 71:2796–98.

SUPPLEMENTARY DATA

Supplementary Table 1. Characteristics of included ARTI episodes (n=758) compared to not included ARTI episodes (n=86) in the study.

	Included ARTI episodes	Not included ARTI episodes[a]	p-value
n	758	86	
Country (n, %)			<0.001
United Kingdom	226 (29.8)	29 (33.7)	
Belgium	234 (30.9)	42 (48.8)	
Netherlands	298 (39.3)	15 (17.4)	
Gender, female (n, %)	410 (54.1)	50 (58.1)	ns
Comorbidity (n, %)	291 (38.4)	29 (33.7)	ns
Age, years (median [IQR])	75 [67 - 80]	75 [68 - 83]	ns
Level of Care Needed (n, %)			<0.001
Hospitalised	4 (0.5)	4 (4.7)	
MA ARTI	170 (22.4)	41 (47.7)	
Non-MA ARTI	576 (76.0)	39 (45.3)	
Not known	8 (1.1)	2 (2.3)	

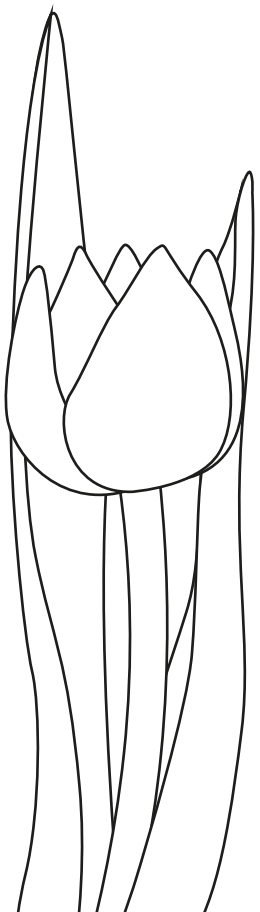
[a]Consisting of missed infections (n= 39) or not tested with both assays (n = 47)
Abbreviations: n, number of episodes; RSV, respiratory syncytial virus; ARTI, acute respiratory tract infection; MA ARTI, Medically Attended ARTI; POCT, point of care test; RT-PCR, reverse transcriptase polymerase chain reaction; ns, not significant.

Supplementary Table 2. Baseline characteristics of participants included in primary analysis.

	Overall (n=561)
Country (n, %)	
United Kingdom	183 (32.6)
Belgium	172 (30.7)
Netherlands	206 (36.7)
Gender, female (n, %)	303 (54.0)
Comorbidity (n, %)	391 (69.8)
Cardiopulmonary disease (n, %)	174 (31.1)
- Cardiac disease (n, %)	118 (21.1)
- Pulmonary disease (n, %)	73 (13.0)
Diabetes (n, %)	59 (10.5)
Age, years (median [IQR])	75 [68- 80]

Abbreviations: n, number of participants.

Goodbye stranger, it's been nice
Hope you find your paradise
(Supertramp – Goodbye Stranger)



Chapter 4

Asymptomatic viral presence in early life precedes recurrence of respiratory tract infections

Roy P. Zuurbier, Debby Bogaert, Wouter A.A. de Steenhuijsen Piters, Kayleigh Arp, Mei Ling J.N. Chu, Elisabeth A.M. Sanders, Marlies A. van Houten

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ABSTRACT

Background. Respiratory tract infections (RTI) in infants are often caused by viruses. Although respiratory syncytial virus (RSV), influenza virus, and human metapneumovirus (hMPV) can be considered the most pathogenic viruses in children, rhinovirus (RV) is often found in asymptomatic infants as well. Little is known about the health consequences of viral presence, especially early in life. We aimed to examine the dynamics of (a)symptomatic viral presence and relate early viral detection to susceptibility to RTIs in infants.

Methods. In a prospective birth cohort of 117 infants, we tested 1,304 nasopharyngeal samples obtained from 11 consecutive regular sampling moments, and during acute RTIs across the first year of life for 17 respiratory viruses by quantitative PCR. Associations between viral presence, viral (sub)type, viral load, viral co-detection, and symptoms were tested by generalized estimating equation (GEE) models.

Results. RV was the most detected virus. RV was negatively associated (GEE: aOR 0.41 [95% CI 0.18-0.92]), and hMPV, RSV, parainfluenza (PIV) 2 and 4, and human coronavirus (HCoV) HKU1 were positively associated with an acute RTI. Asymptomatic RV in early life was, however, associated with increased susceptibility to and recurrence of RTIs later in the first year of life (Kaplan-Meier survival analysis: p=0.022).

Conclusions. Respiratory viruses, including the seasonal HCoVs, are often detected in infants, and are often asymptomatic. Early life RV presence is, though negatively associated with an acute RTI, associated with future susceptibility to and recurrence of RTIs. Further studies on potential ecological or immunological mechanisms are needed to understand these observations.

Keywords:
birth cohort study, respiratory viruses, respiratory tract infections, asymptomatic viral detection, infants

BACKGROUND

Respiratory tract infections (RTI) are a major cause of morbidity and mortality in children worldwide [1,2]. Viruses are the most frequent cause of RTIs in infants and responsible for the high incidence rates during the first 2 years of life [3]. Many respiratory viruses, however, can be considered pathobionts, as they may cause disease, but also colonize the host asymptomatically, and therefore are frequently found in the respiratory tract of asymptomatic young children [4,5]. Human rhinovirus (RV) is reported to be the most frequent cause of RTIs, though respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are responsible for the most severe RTIs at young age [6–10].

Besides their role in RTIs, respiratory viruses have also been suggested to play an important role in the development of recurrent wheeze, asthma and possibly allergic sensitization later in life [11]. Most of these studies have been based on infants hospitalized with these RTIs, typically focusing on lower RTIs and thus more serious illness. In contrast, evidence indicates that half of the first 'infections' with RV might occur asymptomatically [12,13]. Despite that, asymptomatic RV presence in neonates have been associated with activation of airway mucosal immunity, thereby enhancing a type 1 proinflammatory response, which can be predictive of further development to asthma and allergic sensitization [14]. Hence, we thought it important to study symptomatic and asymptomatic presence of respiratory viruses in early life and their effect on later-life health.

In the current study we investigated the presence of 17 respiratory viruses during routine sampling (11 consecutive samples) and during respiratory tract infections (RTI) in a birth cohort of 117 infants over the first year of life. Our aim was to describe the prevalence of viruses across the first year of life, and its relationship with acute and subsequent RTI symptoms in the first year of life.

METHODS

Study population

This study is part of an ongoing Dutch prospective birth cohort aiming to investigate the development of the infant microbiome during health and disease, the Microbiome Utrecht Infant Study (MUIS). In total, 128 healthy term-born infants were enrolled

between December 2012 and June 2014. Details on the study methods have been described elsewhere [15]. Recruitment took place during pregnancy and written informed consent for enrollment was obtained from both parents before birth of the child. The Ethics Committee of Noord Holland, the Netherlands approved the study (M012-015, NH012.394, NTR3986). Data of 117 infants who completed the 1-year follow-up and of which 7 or more nasopharyngeal samples available were used for this study.

Data collection

At baseline, data were collected on prenatal and perinatal characteristics. Samples were obtained using nasopharyngeal (FLOQSwab [484CE]; Copan Diagnostics) swabs. Nasopharyngeal (NP) swabs were collected within 2 hours after birth, at 24 hours, at 7 and 14 days, and at 1, 2, 3, 4, 6, 9 and 12 months during home visits. These routine visits were defined as asymptomatic or routine visits. The swabs were taken according to World Health Organization protocol for nasopharyngeal sampling [16]. Number of experienced RTIs during the first year of life were defined by an extensive survey on health status of the participant, which included RTI symptoms (such as runny nose, shortness of breath, or coughing) experienced since last visit, which was completed during each home visit by parents. The questionnaire was obtained by a trained research team member. Additional home visits were performed, in case of an RTI symptom in combination with fever (38 degrees Celsius or higher) for longer than 6 hours. These symptomatic visits were defined as RTI visits. A RTI visit was planned within 48 hours after start of the fever to collect an additional NP swab. Routine visits were re-located to an RTI visit if participants met these criteria. Mild symptoms could occur during routine visits.

Viral qPCR

Nucleid acids were extracted from one aliquot of 200 µL of NP swab storage medium, using the Purelink™ Viral RNA/DNA Mini Kit (Life Technologies Corporation, Carlsbad, CA, USA). We performed quantitative PCR using primers, probes and PCR assay condition specific for 17 respiratory viruses: adenovirus, parainfluenza virus (PIV) 1–4 [17], human bocavirus [18], human coronavirus (HCoV) OC43, NL63 and 229E [19,20], HCoV HKU1 [21], RSV (A and B) [22,23], hMPV[24], human RV, enterovirus, and influenza virus A [25] and B [26]. The quantitative PCR results were considered positive when the Ct-value was less than 40.

Statistical analysis

Statistical analyses were conducted using R version 4.0.2 within RStudio version 1.2.5. A p-value <0.05 was considered statistically significant. The study had originally been powered based on the abundance and distribution of previously published microbiota data from infants [27], ensuring a power of 0.8 to detect at least significant differences in alpha and beta diversity between groups, as well as differences in abundance of the 25 most important operational taxonomical units (OTUs). Given that we assessed a lower number of outcome measures in a larger cohort compared to the original study, we assume we had sufficient power to detect significant differences between groups. Viral load was studied by using the Ct-values as semiquantitative measure of viral load. Univariable analyses testing differences in viral presence or viral load between routine and RTI visits were done by χ^2 test or Fisher's exact and t-tests, respectively. For longitudinal data analysis we used generalized estimating equations (GEE) with logit or gaussian link to analyze the association between either routine or RTI visits and detection of viruses or viral load, respectively. In these models, individuals were clustered to adjust for repeated measurements. The multivariable model on viral detection and RTI visits included age and all viruses, to adjust for co-detection. Multivariable analysis on the association of viral load and RTI visits was adjusted for age, co-detection and virus type. To assess the association between first virus detection and number of RTIs we stratified our cohort in three susceptibility groups based on the normal distribution, i.e. 0-2, 3-4 and 5-7 RTIs during the first year of life [28] and used a survival analysis (Kaplan-Meier) and a one-way ANOVA analysis to assess statistical significance. Risk factors for early viral detection were analyzed by generalized linear models, including breastfeeding, season of birth, having siblings, and mode of delivery according to previous literature [14].

RESULTS

Patient population

We analyzed in total 1,304 NP swabs from 117 subjects who completed the 1-year follow-up and for whom seven or more samples were available (7-13 samples/subject; 113 subjects providing ≥10 samples). Baseline characteristics of the subjects and samples are summarized in Table, Supplemental Digital Content1. In total, 38% was born by C-section, 46% were being breastfed for at least 3 months with a median duration of 133 days. More than half of the infants had a sibling younger than 5 years

of age. Table, Supplemental Digital Content 2 shows the baseline characteristics stratified by susceptibility groups, i.e., 0-2, 3-4 or 5-7 RTIs reported over the first year of life.

Virus detection

In total, 638 viruses were detected from both routine samples and samples obtained during acute RTI visits. Overall, 36.8% (480/1304) of the NP swabs were positive for at least one virus, for the routine visits this was 34.4% (429/1246) versus 88% (51/58) in acute RTI samples (Table, Supplemental Digital Content 3). Figure 1 shows the detection of respiratory viruses per timepoint. Viral detection increased strongly with age, from 4% (5/113) of the infants in the first day of life to 68% (76/112) at 12 months of life. RV was the most commonly detected virus (319/1304 (24.4% of all samples)), and detected as early as <2 hours after birth (4 cases). In almost all participants (94%) RV was detected at least once over the first year of life. Only two infants showed no virus detection during the entire follow-up period. These infants also reported only one or two episodes of RTIs during the first year of life. Table, Supplemental Digital Content 3 shows viruses detected during routine visits versus RTI visits. Following RV (24.6%; 306/1246) and adenoviruses (5.4%; 67/1246), HCoV were the third most detected group of viruses in routine swabs, i.e., in 4.3% (53/1246). HCoVs were detected at least once in 40% (n= 47) of the participants during the first year of life.

Co-detection

Detection of 2 or more viruses (co-detection) was observed in 10% (125/1304) of all the swabs, and only from month 1 onward (Figure, Supplemental Digital Content 4). In co-detection, RV (75%; 94/125) and adenovirus (47%; 59/125) were the most frequently observed, and both were relatively more commonly detected in co-infections compared to single detection (χ^2 test $p=0.022$ and $p<0.001$, respectively). Multivariable generalized estimating equation (GEE) analysis showed a significant positive association for the presence of two or more viruses compared to a single virus with older age ($p<0.001$), and daycare attendance ($p<0.001$; Table, Supplemental Digital Content 5). Having siblings (<5y) and RTI status did not show a statistical significant effect.

Respiratory tract infections

In total, the study team performed 58 RTI visits for 42 of the participants Of these swabs, 51 were positive for at least one virus, which was significantly more common compared to routine swabs, also after correction for age (aOR: 5.33 [95% CI 2.42-11.71]; p<0.001 (GEE); Table, Supplemental Digital Content 6). Adenovirus (29%) was most commonly detected during RTI, followed by RSV and RV (both 22%). With respect to the HCoVs, only HCoV-HKU1 was more often found in RTI samples compared to routine sampling (9% vs 1%, Fisher exact test p<0.001). Figure 2 shows the proportion of individual viruses during RTI and routine sampling moments. Co-detection occurred in 41% of the RTI episodes. RV and bocavirus, were significantly more often co-detected compared to single detection during RTI episodes (χ^2 test: p= 0.001 and p=0.002, respectively).

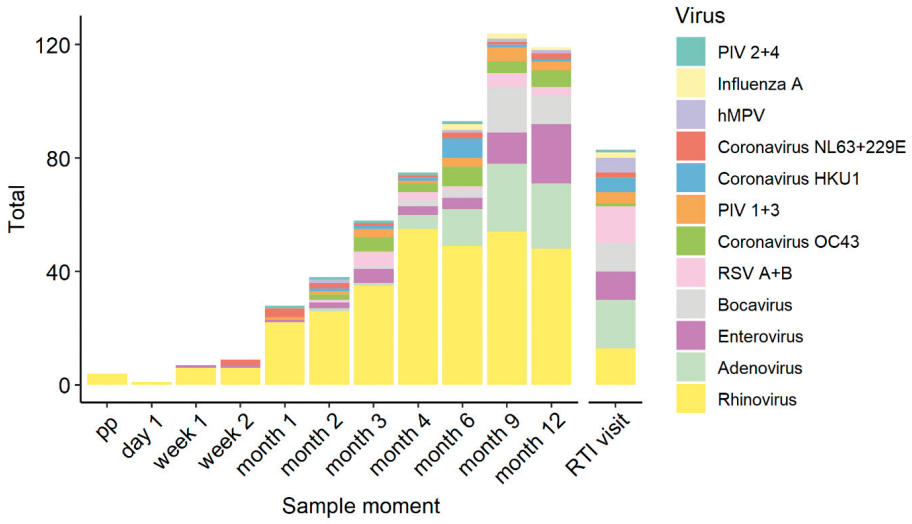

Figure 1. Viruses per sample moment
Total detected viruses per timepoint (n=638). Legend shows color per virus. Right column shows viruses detected during a RTI visit.
hMPV: human metapneumovirus; PIV: parainfluenza; pp = post-partum (swab taken within 2 hours after birth); RSV: respiratory syncytial virus.

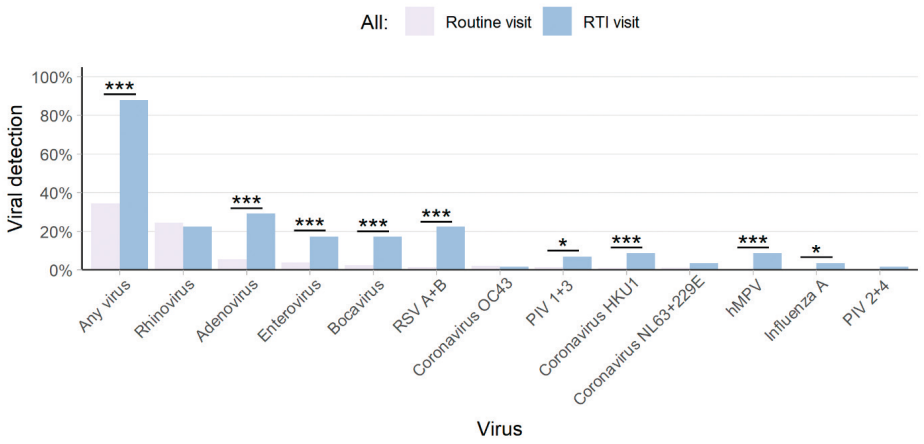

Figure 2. Viral PCR positivity in routine and RTI visits.
The proportions of qPCR respiratory virus detections for RTI (symptomatic) visits (black, n=58) and routine (asymptomatic) visits (grey, n=1,246). P values were calculated with chi-square test or Fisher's exact. Significance symbols: *** = p<0.001; ** = p<0.01; * = p<0.05.

Multivariable longitudinal analysis (including age) was used to study the association between individual viruses and symptomatic infections (Table 1). We found that hMPV, RSV, and PIV 2 and 4, were most significantly related to symptomatic RTIs (aOR > 10, Table 1). Enterovirus, and adenoviruses were also significantly associated with symptomatic RTIs, however showing a lower odds ratio (Table 1). Regarding HCoVs, a significant positive association was only found between HCoV-HKU1 (aOR: 9.09 [95% CI 2.78-29.77]) and RTIs. We found a negative association between RV and symptomatic RTIs (aOR: 0.41 [95% CI 0.18 – 0.92]; p = 0.031). For influenza (A), PIV 1 and 3, HCoV (OC43 and, NL63 and 229E), and bocavirus no significant associations with either RTIs or routine visits were found.

Viral co-detection was found more commonly in virus positive samples during RTI visits compared to routine visits in both univariable (47%; 24/51 vs 24%; 101/429, χ^2 test p<0.001) and multivariable analysis (GEE: aOR: 2.03 [95% CI 1.08-3.79], Table, Supplemental Digital Content 7), after adjusting for age and virus type.

Table 1. Odds ratio for RTI association per virus type. Odds ratios for respiratory viruses on association with an RTI episode (n=1,304).

Virus	**Crude OR** [95% CI]	**aOR** [95% CI]	**95% CI interval**
hMPV	25.172* [6.27 – 101.06]	28.65*	5.39 – 152.15
RSV A+B	17.57* [8.25 – 37.41]	13.24*	4.44 – 39.48
PIV 2+4	4.10 [0.50 – 33.85]	11.37*	1.23 – 104.94
HCoV HKU1	8.03* [2.67 – 24.15]	9.09*	2.78 – 29.77
Influenza A	8.25* [1.63 – 41.88]	5.43	0.95 – 31.12
Enterovirus	4.48* [2.14 – 9.39]	4.82*	1.73 – 13.42
Adenovirus	6.60* [3.61 – 12.09]	3.89*	1.57 – 9.62
PIV 1+3	4.57* [1.43 – 14.57]	3.14	0.63 – 15.57
Bocavirus	7.05* [3.37 – 14.77]	2.29	0.70 – 7.49
HCoV NL63 and 229E	3.06* [0.76 – 12.31]	1.69	0.19 – 15.16
HCoV OC43	0.73 [0.11 – 4.85]	0.77	0.11 – 5.14
RV	0.81 [0.48 – 1.48]	0.41*	0.18 – 0.92

All respiratory viruses were used in this multivariable model including age effect and adjusted for virus type. Odds ratios (OR) and 95% confidence interval were determined by multivariate generalized estimating equations.* = p<0.05. aOR: adjusted odds ratio; CI: confidence interval; HCoV: human coronavirus; hMPV: human metapneumovirus; OR: Odds ratio; PIV: parainfluenza virus; RSV: respiratory syncytial virus; RV: rhinovirus.

Time to viral infection and health consequences

Since virus detection early in life could be a risk factor for respiratory morbidity [14], we studied this in our cohort. The median number of days to first virus detection was 92 [IQR 33 – 124] days. In more than 75% of cases we observed RV as the first virus detected. Survival analyses showed a significant association between RTI susceptibility groups and age at first virus detection (median age 92, 63 and 62 for 0-2, 3-4 and 5-7 RTIs, respectively, p = 0.027; Figure, Supplemental Digital Content 8). This association was also seen for days to first RV identification (p = 0.022; Figure 3). To confirm these results a one-way ANOVA was performed (Figure, Supplemental Digital Content 9, p=0.03). No significant association was found for days to first non-RV detection and the association between RTI susceptibility groups. Using multivariable linear model, we found that risk factors for early detection of RV were being born in winter (p=0.01), having young (<5 years old) siblings (p<0.01), where mode of delivery or cumulative days of breastfeeding were not significantly associated with early RV detection (Table, Supplemental Digital Content 10). Table, Supplemental Digital Content 11 shows the baseline characteristics stratified by rhinovirus detection before three months of age and after.

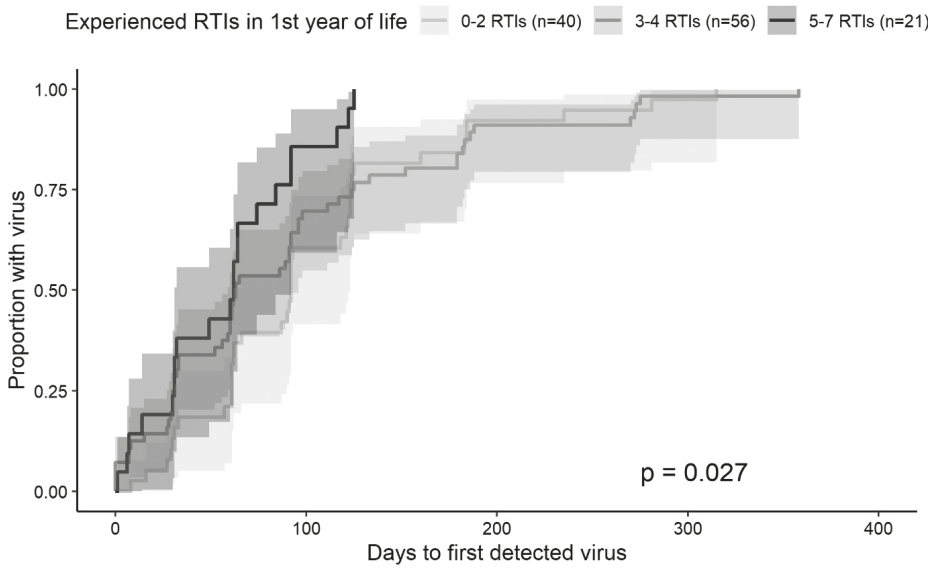

Figure 3. Survival curve showing days to first RV detection and experienced RTIs during first year of life.

X-axis show days to first detected RV infection in first year of life (n=117). Colored lines show different RTI susceptibility groups in the first year of life. The p-value was calculated by Kaplan-Meier analysis (p = 0.022).

Viral load

Viral load was measured as reciprocal of Ct-value, i.e., a high Ct-value represents low viral load. The mean overall Ct-value for all positive viral qPCRs was 25.5. During RTIs the viral load was significantly higher for HCoV-OC43 and HKU1, PIV 1 and 3, enterovirus, bocavirus and adenovirus compared to routine swabs (Table, Supplemental Digital Content 12, Figure 4). Using multivariable longitudinal analysis, we observed a significant higher load in RTI samples, with a beta-coefficient of 3.6 Ct-values (GEE: $p<0.001$), when adjusted for age, viral co-detection and virus type. Viral load did not vary with age or viral co-detection in this model (Figure, Supplemental Digital Content 13; Figure, Supplemental Digital Content 14).

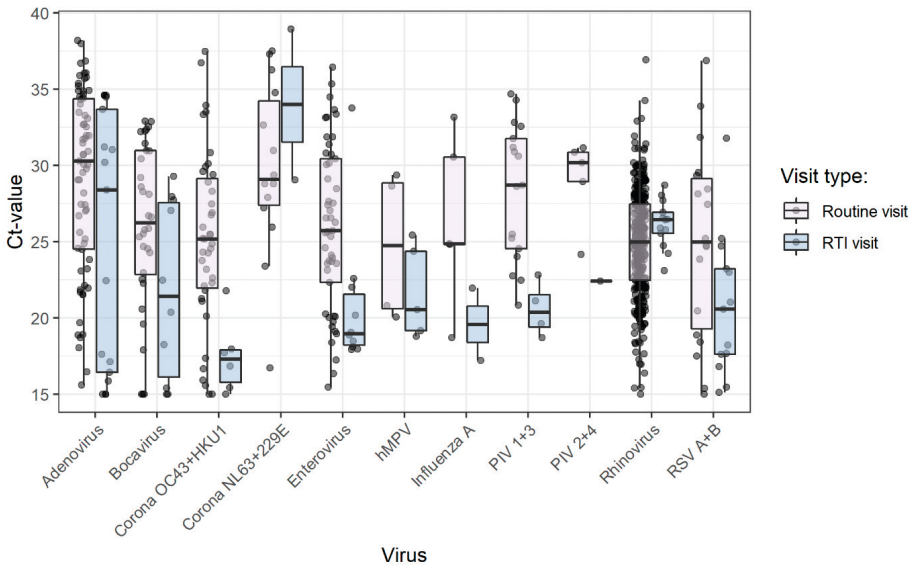

Figure 4. Ct-value per virus, grouped by RTI and routine visits.
Ct-values of all viruses detected in samples, grouped by routine and RTI visits (n=638). Boxplots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line).

DISCUSSION

In this longitudinal birth cohort study, we studied the presence of common respiratory viruses over the first year of life in relation to symptomatology as well as the development of (recurrent) RTIs. Respiratory viruses were observed from early in life on, and were often asymptomatic. We most commonly detected RV from the first days of life on, which was negatively associated with RTIs when compared to asymptomatic episodes. In contrast, detection of hMPV, PIV 2 and 4, and RSV were highly associated with RTI. Notably, influenza viruses were rarely observed, which however might be explained by a low endemicity of influenza in general in the two recruitment years. Adding to that, lack of significance of other viruses could be due to low numbers of detections. The negative association of RV with RTIs has also previously been found [5,29]. Interestingly, RV is often found in combination with other viruses in RTIs in our cohort, suggesting merely a potentiating role or a bystander effect in the etiology of RTIs. However, additionally, despite the nonpathogenic behavior of RV, early detection of the virus was associated with recurrence of RTIs in the first year of life.

Interestingly, we detected the first respiratory viruses in the nasopharynx of infants as early as in the first days of life. In the first period of life, these infections occurred mostly asymptomatically. No information is available on parents having a RTI, however it is very common that adults are asymptomatically colonized with rhinovirus and can transmit this within their family [30,31]. Our findings on timing of first detected viruses, are in line with a birth cohort by Sarna et al., who collected weekly nose swabs during the first two years of life, and showed a median age of first virus detection of 2.9 months [12], versus a median age of 3.0 in our study.

Importantly, our findings show that early detection of respiratory viruses, especially detection of asymptomatic RV, was related to an increased number of RTIs experienced in the first year of life. We hypothesize this could be the result of early interaction of the virus with the host's immune system and can lead to immunomodulation. This has previously been postulated by Wolsk et al. [14] who showed that the presence of picornaviruses (including RV and enterovirus) in the first four weeks of life were related to an increased release of T-helper 2 mucosal immune response. They therefore hypothesized that RV presence in the first year of life can be an external trigger for wheezing and asthma development later in life. Why viral presence early in life occur mostly asymptomatically is also important to understand; this might be due to the protective effects of maternal antibodies, the composition and immune modulatory role of the microbiome, or the immaturity of the immune system including a degree of early life immune tolerance. Until now, little is known about the effect of asymptomatic viral presence in early in life, therefore further mechanistic studies are warranted.

We also commonly detected seasonal HCoVs in our cohort. This study was executed before the SARS-CoV-2 pandemic, therefore SARS-CoV-2 could not have been detected yet. However, SARS-CoV-2 is a beta-HCoV and belongs to the B lineage. HCoV-OC43 and HKU1 are also beta-HCoV and the closest human HcoVs related to SARS-CoV-2 [32]. In our study, we found in 41% of the participants at least once a HCoV during the first year of life. In total, 3% of all routine samples were positive for HCoV-OC43 or HKU1, and 10% of RTI samples, which is in line with previous studies, showing similar though slightly higher percentages (6-8% versus 4.3%) of asymptomatic HCoV detection (OC43, NL63, 229E) [13,33], which is likely a result of slight differences in age and season of sampling. Of the seasonal HcoVs, HCoV-HKU1 was the only HCoV found to be positively associated with RTI in our study. This is in

contrast with a similar cohort study executed in children ntil the age of 2 in Australia, where HCoV-NL63 and HCoV-OC43 were suggested to be more pathogenic [7]. A prospective surveillance study showed that HCoV is not often found in hospitalized young children and were mostly found as co-detection [34]. Another report showed that a higher rate of RSV co-detection distinguished children with HCoV-associated LRTI from asymptomatic HCoV carriers and also from children with a non-HCoV-associated LRTI [35]. This is in line with our findings, showing a positive association between viral co-detection with HcoVs and symptomatic RTIs (aOR: 3.30) (Table, Supplemental Digital Content 15).

Strengths of this study is the prospective birth cohort set-up, allowing to investigate viral dynamics and its relation with RTIs over the first year of life. Samples were collected at pre-specified intervals in the first year of life and during RTIs. The study visits provided us with a large amount of longitudinally obtained samples during both routine visits as well as during symptomatic episodes. We focused on nasopharyngeal samples taken by trained research nurses and physicians, ensuring quality and consistency of sampling. In contrast to anterior nasal swabs or swabs taken by parents, nasopharyngeal swabs have proven to be highly sensitive to detect respiratory viruses [29,36]. Another strength is that we used qPCR to identify a broad panel of 17 common respiratory viruses, and providing us with semi-quantitative data.

There are some limitations in our study. First, parents were asked to contact the research team in case of a febrile RTI. Therefore, it is likely we have missed some of these episodes. However, efforts were made to obtain detailed information on all RTIs, to ensure reporting bias was minimal. In addition, if an infant was suffering of a febrile RTI during a routine visit, it was considered an RTI visit instead. Adding to that, the data on viruses identified were described in percentages and not absolute data. We therefore do not think this limitation has a major impact on the results. Second, even though we sampled frequently during the first months of life, intervals increased to a maximum of 3 months later in the first year of life. Therefore, we likely have missed episodes of asymptomatic viral presence in between sampling moments, which will lead to an underestimation of prevalence numbers at later age. In addition, the risk of recall bias increased regarding the use of retrospective questionnaires about the occurrence respiratory infections in the study group. Third, number of RTIs was low, resulting in a wide confidence interval. Fourth, exposure to

respiratory viruses seems to be a risk factor for both early detection and increased susceptibility to RTIs, such as having young siblings. This could be a confounding factor in our analysis. However, having young siblings was not statistically different between the three susceptibility groups. We did not include daycare as risk factor for early detection of RV into our model, considering median age at start daycare was 122 days and median age of first RV detection was 92 days. Therefore, most infants already had their first RV detection before going to daycare. Fifth, the original study was enriched for C-section born infants that were otherwise healthy, resulting in 38% of the participants born by C-section. Though in the Netherlands C-sections are executed in approximately 10 to 15% of all deliveries, in many countries this is significantly higher. Therefore, this cohort can be regarded as representative for the general community. Last, although inferring viral loads from Ct values is convenient, it has its limitations and provides only a semiquantitative estimate. It should therefore interpret with caution.

In conclusion, respiratory viruses are often detected in infants, and are often present asymptomatically, even very early in life. Especially, RV was detected in almost a quarter of all samples and was less often found during RTI visits compared to routine sampling. However, importantly, we found an positive association of early life RV detection with subsequent susceptibility to RTIs in the first year of life, suggesting RV may affect either the microbial ecology or act as immunomodulator. Understanding the biological mechanisms underpinning this association are important to understand whether and how this may lead to short and long-term sequelae, including wheezing illness and asthma.

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REFERENCES

1. Vos T, Barber RM, Bell B, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;386(9995):743–800.
2. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*. 2016;388(10063):3027–3035.
3. Jain S, Williams DJ, Arnold SR, et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Children. *N Engl J Med*. 2015;372(9):835–845.
4. Man WH, De Steenhuijsen Piters WAA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. *Nat Rev Microbiol*. 2017;15(5):259–270.
5. Van Den Bergh MR, Biesbroek G, Rossen JWA, et al. Associations between Pathogens in the Upper Respiratory Tract of Young Children: Interplay between Viruses and Bacteria. Rogers LK, ed. *PLoS One*. 2012;7(10):e47711.
6. Shi T, McLean K, Campbell H, et al. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta–analysis. *J Glob Health*. 2015;5(1):1-10.
7. Sarna M, Lambert SB, Sloots TP, et al. Viruses causing lower respiratory symptoms in young children: findings from the ORChID birth cohort. *Thorax*. 2018;73(10):969–979.
8. van der Zalm MM, Uiterwaal CSPM, Wilbrink B, et al. Respiratory Pathogens in Respiratory Tract Illnesses During the First Year of Life. *Pediatr Infect Dis J*. 2009;28(6):472–476.
9. Kusel MMH, de Klerk NH, Holt PG, et al. Role of Respiratory Viruses in Acute Upper and Lower Respiratory Tract Illness in the First Year of Life. *Pediatr Infect Dis J*. 2006;25(8):680–686.
10. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol*. 2010;82(7):1282–1290.
11. Jartti T, Gern JE, Finland M. Role of viral infections in the development and exacerbation of asthma in children.
12. Sarna M, Ware RS, Lambert SB, et al. Timing of First Respiratory Virus Detections in Infants: A Community-Based Birth Cohort Study. *J Infect Dis*. 2018;217(3):418–427.
13. van der Zalm MM, van Ewijk BE, Wilbrink B, et al. Respiratory Pathogens in Children with and without Respiratory Symptoms. *J Pediatr*. 2009;154(3):396-400.e1.
14. Wolsk HM, Følsgaard N V, Birch S, et al. Picornavirus-Induced Airway Mucosa Immune Profile in Asymptomatic Neonates. *J Infect Dis*. 2016;213(8):1262–1270.
15. Bosch AATM, Levin E, van Houten MA, et al. Development of Upper Respiratory Tract Microbiota in Infancy is Affected by Mode of Delivery. *EBioMedicine*. 2016;9:336–345.
16. O'BRIEN KL, NOHYNEK H. Report from a WHO working group: standard method for detecting upper respiratory carriage of Streptococcus pneumoniae. *Pediatr Infect Dis J*. 2003;22(2):133–140.
17. van de Pol AC, van Loon AM, Wolfs TFW, et al. Increased Detection of Respiratory Syncytial Virus, Influenza Viruses, Parainfluenza Viruses, and Adenoviruses with Real-Time PCR in Samples from Patients with Respiratory Symptoms. *J Clin Microbiol*. 2007;45(7):2260–2262.
18. Allander T, Jartti T, Gupta S, et al. Human Bocavirus and Acute Wheezing in Children. *Clin Infect Dis*. 2007;44(7):904–910.

19. van Elden LJR, van Loon AM, van Alphen F, et al. Frequent Detection of Human Coronaviruses in Clinical Specimens from Patients with Respiratory Tract Infection by Use of a Novel Real-Time Reverse-Transcriptase Polymerase Chain Reaction. *J Infect Dis*. 2004;189(4):652–657.
20. Zlateva KT, Coenjaerts FEJ, Crusio KM, et al. No novel coronaviruses identified in a large collection of human nasopharyngeal specimens using family-wide CODEHOP-based primers. *Arch Virol*. 2013;158(1):251–255.
21. Loens K, van Loon AM, Coenjaerts F, et al. Performance of Different Mono- and Multiplex Nucleic Acid Amplification Tests on a Multipathogen External Quality Assessment Panel. *J Clin Microbiol*. 2012;50(3):977–987.
22. Van Elden LJR, Van Loon AM, Van Der Beek A, et al. Applicability of a Real-Time Quantitative PCR Assay for Diagnosis of Respiratory Syncytial Virus Infection in Immunocompromised Adults. *J Clin Microbiol*. 2003;41(9):4378–4381.
23. van Elden LJR, van Loon AM, van der Beek A, et al. Applicability of a Real-Time Quantitative PCR Assay for Diagnosis of Respiratory Syncytial Virus Infection in Immunocompromised Adults. *J Clin Microbiol*. 2005;43(8):4308–4308.
24. Maertzdorf J, Wang CK, Brown JB, et al. Real-Time Reverse Transcriptase PCR Assay for Detection of Human Metapneumoviruses from All Known Genetic Lineages. *J Clin Microbiol*. 2004;42(3):981–986.
25. Hoek RAS, Paats MS, Pas SD, et al. Incidence of viral respiratory pathogens causing exacerbations in adult cystic fibrosis patients. *Scand J Infect Dis*. 2013;45(1):65–69.
26. Ward CL, Dempsey MH, Ring CJA, et al. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. *J Clin Virol*. 2004;29(3):179–188.
27. Bogaert D, Keijser B, Huse S, et al. Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PLoS One*. 2011;6(2).
28. Bosch AATM, Piters WAA de S, van Houten MA, et al. Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health Consequences. A Prospective Cohort Study. *Am J Respir Crit Care Med*. 2017;196(12):1582–1590.
29. Man WH, van Houten MA, Mérelle ME, et al. Bacterial and viral respiratory tract microbiota and host characteristics in children with lower respiratory tract infections: a matched case-control study. *Lancet Respir Med*. 2019;7(5):417–426.
30. Peltola V, Waris M, Osterback R, et al. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis*. 2008;197(3):382–389.
31. Zlateva KT, van Rijn AL, Simmonds P, et al. Molecular epidemiology and clinical impact of rhinovirus infections in adults during three epidemic seasons in 11 European countries (2007–2010). *Thorax*. 2020;75(10):882–890.
32. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*. March 2020:1–9.
33. Chonmaitree T, Alvarez-Fernandez P, Jennings K, et al. Symptomatic and Asymptomatic Respiratory Viral Infections in the First Year of Life: Association With Acute Otitis Media Development. *Clin Infect Dis*. 2015;60(1):1–9.
34. Calvo C, Alcolea S, Casas I, et al. A 14-year Prospective Study of Human Coronavirus Infections in Hospitalized Children: Comparison With Other Respiratory Viruses. *Pediatr Infect Dis J*. 2020;39(8):653–657.
35. de Koff EM, van Houten MA, Sanders EAM, et al. Severity of Respiratory Infections With Seasonal Coronavirus Is Associated With Viral and Bacterial Coinfections. *Pediatr Infect Dis J*. 2021;40(1):e36–e39.

36. Munywoki PK, Hamid F, Mutunga M, et al. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real-time PCR using nasopharyngeal flocked swabs. *J Clin Microbiol*. 2011;49(9):3365–3367.

SUPPLEMENTAL DIGITAL CONTENT

Table, Supplemental Digital Content 1. Baseline characteristics for all participants.

	Overall (n=117)
Sex, male (n (%))	60 (51)
Season of birth (n (%))	
Winter	24 (21)
Spring	29 (25)
Summer	41 (35)
Autumn	23 (20)
Birth mode, vaginally (n (%))	72 (62)
Birth weight (mean (SD))	3536.31 (482.80)
Gestational age (mean (SD))	39.51 (1.13)
High education level of parents (n (%))	88 (75)
Inhouse smoking (n (%))	3 (3)
Breastfeeding, (n (%)) No breastfeeding <3 months 3-6 months >6 months	28 (24) 33 (28) 19 (16) 37 (32)
Siblings <5y (n (%))	67 (57)
Daycare attendance (n (%))	82 (70)
Antibiotics for respiratory tract infection in first year of life (n (%))	33 (28)

Data was obtained by questionnaires at all routine and RTI visits. Breastfeeding was nonexclusive. High educational level was defined as one of the parents went to university of applied sciences or research university.

Table, Supplemental Digital Content 2. Baseline characteristics stratified by susceptibility groups.

Susceptibility groups (n)	0 – 2 RTIs (n = 40)	3 – 4 RTIs (n = 56)	5 – 7 RTIs (n = 21)	p-value
Sex, male (n (%))	25 (63)	23 (41)	12 (57)	0.10
Season of birth (n (%))				0.55
Winter	5 (13)	14 (25)	5 (24)	
Spring	11 (28)	11 (20)	7 (33)	
Summer	14 (35)	20 (36)	7 (33)	
Autumn	10 (25)	11 (20)	2 (10)	
Birth mode, vaginally (n (%))	28 (70)	33 (59)	11 (52)	0.35
Birth weight (mean (SD))	3498.25 (510.75)	3558.30 (508.36)	3550.14 (357.95)	0.83
Gestational age (mean (SD))	39.72 (1.22)	39.36 (1.13)	39.50 (0.95)	0.31
High education of parents (n (%))	31 (78)	43 (77)	14 (67)	0.60
Inhouse smoking (n (%))	0 (0.0)	3 (5)	0 (0.0)	0.19
Breastfeeding, (n (%)) No breastfeeding <3 months 3-6 months >6 months	7 (18) 9 (23) 9 (23) 15 (38)	16 (29) 15 (27) 8 (14) 17 (30)	27 (24) 9 (43) 2 (10) 5 (24)	<0.001
Siblings <5y (n (%))	17 (43)	35 (63)	15 (71)	0.05*
Daycare attendence (n (%))	24 (60)	43 (77)	15 (71)	0.21
Antibiotics for respiratory tract infection in first year of life (n (%))	6 (15)	17 (31)	8 (38)	0.11
RTI episode, at least one (n(%))	9 (22.5)	19 (33.9)	14 (66.7)	0.003

Data was obtained by questionnaires at all routine and RTI visits. Breastfeeding was nonexclusive. High educational level was defined when one of the parents went to university of applied sciences or research university. P-values were determined by chi-square tests or one-way ANOVA test.
* non-significant (p = 0.052)

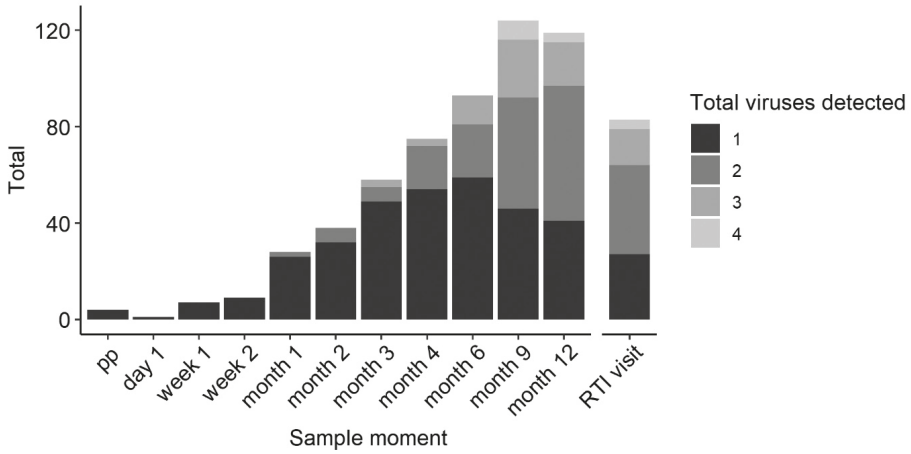
Table, Supplemental Digital Content 3. Viral PCR positivity in routine visits and RTI visits. The proportions of qPCR respiratory virus detections for RTI (symptomatic) visits (n=58) and routine (asymptomatic) visits (n=1,246).

	Routine visit	RTI visit	p-value
n	*1246*	*58*	
Influenza A (%)	5 (0.4)	2 (3.4)	0.04
Influenza B (%)	0 (0.0)	0 (0.0)	NA
RSV A+B (%)	18 (1.4)	13 (22.4)	<0.001
HCoV NL63+229E (%)	14 (1.1)	2 (3.4)	0.16
HCoV OC43 (%)	27 (2.2)	1 (1.7)	1.00
HCoV HKU1 (%)	12 (1.0)	5 (8.6)	<0.001
HCoV total	*53 (4.3)*	*7 (12.1)**	*0.01*
PIV 1+3 (%)	17 (1.4)	4 (6.9)	0.01
PIV 2+4 (%)	5 (0.4)	1 (1.7)	0.24
PIV total	*22 (1.8)*	*5 (8.6)*	*0.002*
hMPV (%)	4 (0.3)	5 (8.6)	<0.001
Adenovirus (%)	67 (5.4)	17 (29.3)	<0.001
Bocavirus (%)	32 (2.6)	10 (17.2)	<0.001
RV (%)	306 (24.6)	13 (22.4)	0.83
Enterovirus (%)	49 (3.9)	10 (17.2)	<0.001
Total viruses (%)			*<0.001*
0	817 (65.6)	7 (12.1)	
1	328 (26.3)	27 (46.6)	
2	78 (6.3)	18 (31.0)	
3	20 (1.6)	5 (8.6)	
4	3 (0.2)	1 (1.7)	
Codetection (%)	101 (8.1)	24 (41.4)	<0.001
At least one virus detected (%)	429 (34.4)	51 (87.9)	<0.001

P-values were determined by chi-square tests or Fisher's exact.

*: one sample was positive for both HCoV OC43 and HCoV NL63.

HCoV: human coronavirus; hMPV: human metapneumovirus; PIV: parainfluenza virus; RSV: respiratory syncytial virus; RV: rhinovirus



Figure, Supplemental Digital Content 4. Number of co-detections per timepoint.
Total viruses detected per timepoint of routine visits and RTI visits (n=638). Legend shows number of co-detections. pp = post-partum (swab taken within 2 hours after birth).

Table, Supplemental Digital Content 5. Odds ratio for viral codetection. Odds ratios for samples taken during routine and RTI visits and association with viral codetection in virus positive samples (n=638).

Variable	**Crude OR** [95% CI]	**aOR** [95% CI]
Age, months	1.02* [1.02 – 1.03]	1.02* [1.01 – 1.02]
RTI visit	2.78* [1.70 – 4.53]	3.13 [0.93 – 10.56]
Having siblings (<5 years of age)	0.86 [0.61 – 1.23]	1.11 [0.74 – 1.67]
Daycare attendance	4.89* [3.40 – 7.05]	2.86* [1.92 – 4.26]

Odds ratios (OR) and 95% confidence interval were determined by multivariate generalized estimating equations. * = $p < 0.05$
aOR: adjusted odds ratio; CI: confidence interval; RTI: respiratory tract infection; OR: odds ratio.

Table, Supplemental Digital Content 6. Odds ratio for viral detection. Odds ratios for samples taken during routine and RTI visits and association with viral detection (n=1,304).

Variable	**Crude OR** [95% CI]	**aOR** [95% CI]
Age, days	1.02* [1.02 – 1.03]	1.02* [1.02 – 1.02]
RTI visit	8.20* [4.31 – 15.61]	5.33* [2.42 – 11.71]

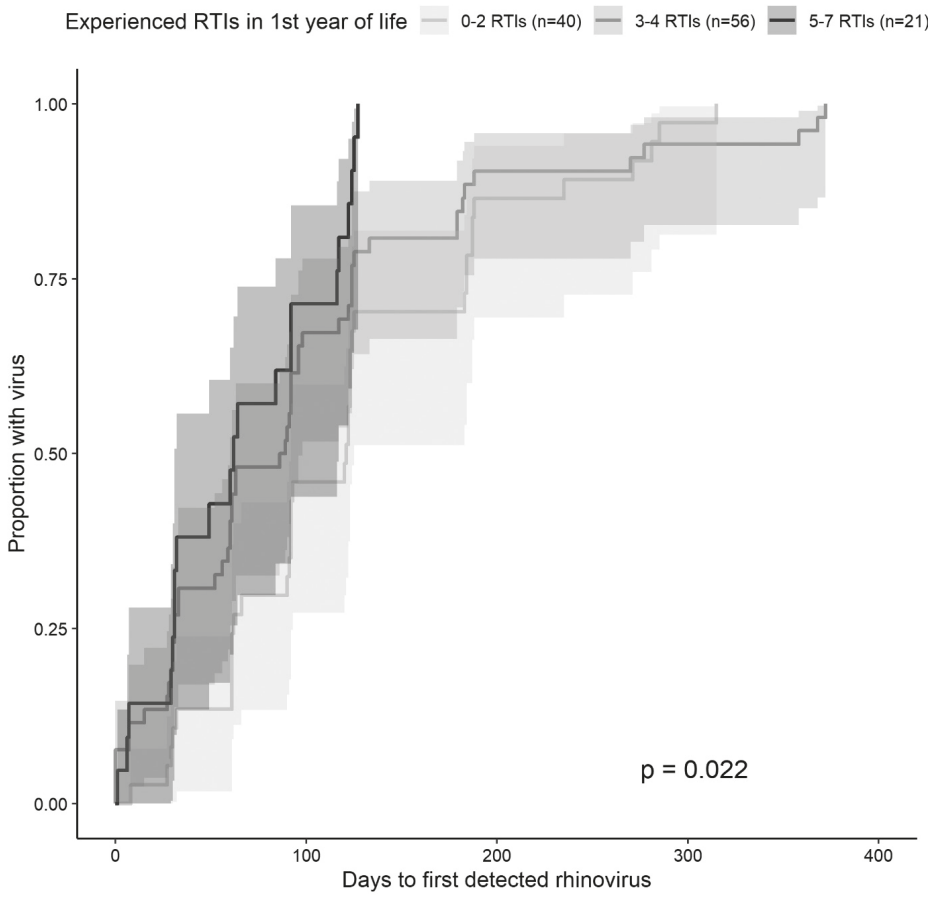
Odds ratios (OR) and 95% confidence interval were determined by multivariate generalized estimating equations.* = $p<0.001$
aOR: adjusted odds ratio; CI: confidence interval; RTI: respiratory tract infection; OR: odds ratio.

Table, Supplemental Digital Content 7. Odds ratio for viral codetection. Odds ratios for samples taken during routine and RTI visits and association with viral codetection in virus positive samples (n=638).

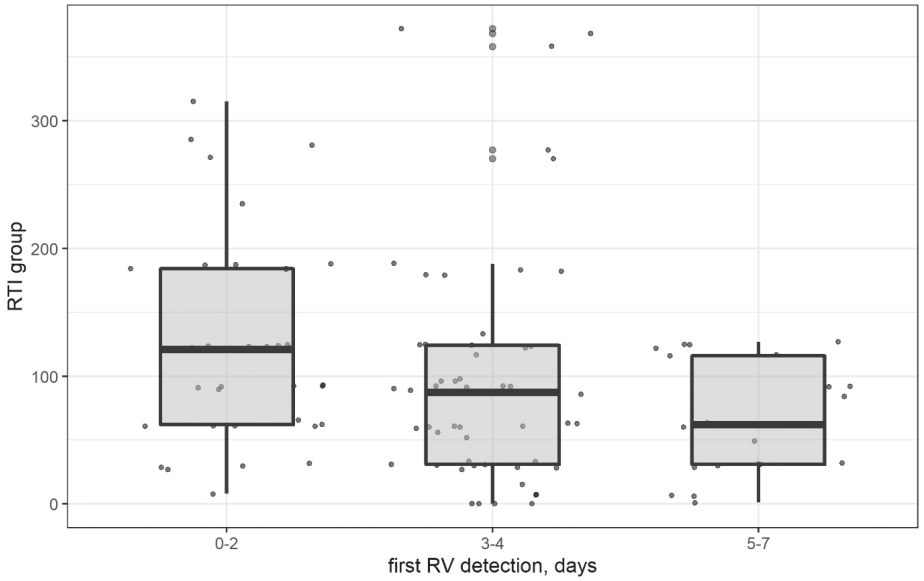
Variable	**Crude OR** [95% CI]	**aOR** [95% CI]
RTI visit	2.77* [1.70 – 4.53]	2.03* [1.08 – 3.79]
Bocavirus	0.95 [0.43 – 2.10]	0.82 [0.36 – 1.90]
HCoV OC43 and HKU1	0.29* [0.13 – 0.64]	0.39* [0.17 – 0.91]
HCoV NL63 and 229E	0.33* [0.11 – 0.99]	0.82 [0.29 – 2.37]
Enterovirus	0.59 [0.32 – 1.20]	0.62 [0.29 – 1.36]
hMPV	0.54 [0.13 – 2.17]	0.46 [0.08 – 2.51]
Influenza A	1.07 [0.19 – 5.90]	1.14 [0.18 – 7.17]
PIV 1+3	0.70 [0.26 – 1.83]	0.98 [0.34 – 2.86]
PIV 2+4	0.09* [0.01 – 0.90]	0.26 [0.02 – 2.90]
RV	0.17* [0.10 – 0.31]	0.30* [0.17 – 0.53]
RSV A+B	0.68 [0.29 – 1.59]	0.85 [0.32 – 2.27]

Odds ratios (OR) and 95% confidence interval were determined by multivariate generalized estimating equations, including age effect. * = $p < 0.05$

aOR: adjusted odds ratio; CI: confidence interval; HCoV: human coronavirus; hMPV: human metapneumovirus; OR: Odds ratio; PIV: parainfluenza virus; RSV: respiratory syncytial virus; RV: rhinovirus;



Figure, Supplemental Digital Content 8. Survival curve showing the association between days to first virus detection and amount of experienced RTIs during first year of life.
X-axis show days to first detected virus in first year of life (n=117). Colored lines show different RTI susceptibility groups in the first year of life. The p-value was calculated by Kaplan-Meier analysis (p = 0.027).



Figure, Supplemental Digital Content 9. Boxplots showing days to first RV detection per experienced RTIs during first year of life groups.
X-axis shows different RTI susceptibility groups in the first year of life. Y-axis shows days to first detected rhinovirus detection in first year of life (n=117). The p-value was calculated by one-way ANOVA analysis (p = 0.03).

Table, Supplemental Digital Content 10. Multivariable model on risk factors for early RV detection. Risk factors for early RV detection in days (n = 117). Generalized linear model was used to calculate estimate coefficients and p-values.

Variable	Estimate coefficient	Standard error	t-value	P-value
Intercept	201.99	25.08	8.05	<0.001
Season of birth				
Winter	-71.20	27.36	-2.60	0.01
Spring	6.82	26.07	0.26	0.79
Summer	-32.51	24.48	-1.33	0.19
Autumn	Ref	Ref	Ref	Ref
Having siblings (<5 years of age)	-61.15	17.81	-3.43	<0.001
Mode of delivery, vaginally	-25.41	18.42	-1.38	0.17
Cumulative breastfeeding days	-0.07	0.07	-1.08	0.28

RV: rhinovirus

Table, Supplemental Digital Content 11. Baseline characteristics stratified by early rhinovirus detection.

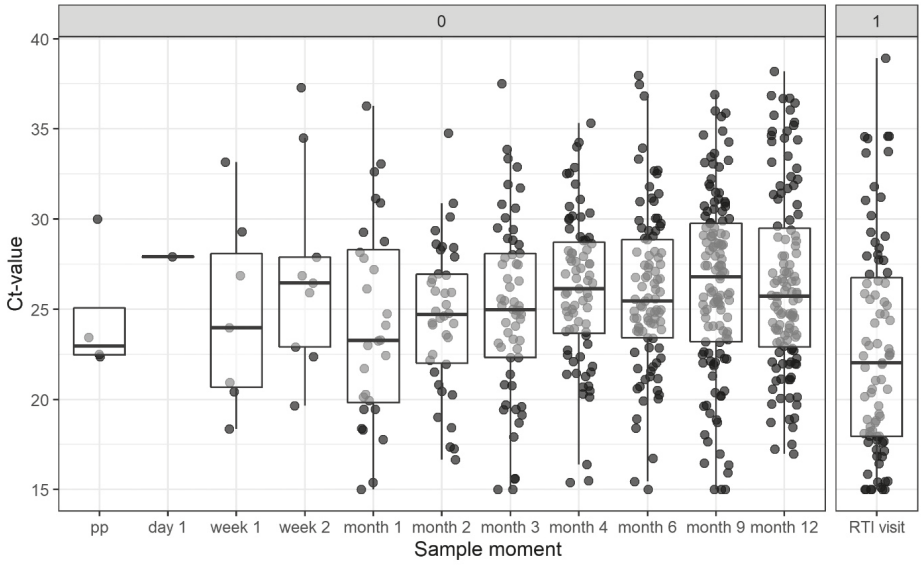
First rhinovirus detection	Before 3 months of age (n = 55)	After 3 months of age* (n = 62)	p-value
Sex, male (n (%))	25 (46)	35 (57)	0.32
Season of birth (n (%))			0.01
Winter	14 (26)	10 (16)	
Spring	7 (13)	22 (36)	
Summer	25 (46)	16 (26)	
Autumn	9 (16)	14 (23)	
Birth mode, vaginally (n (%))	36 (66)	36 (58)	0.53
Birth weight (mean (SD))	3568.96 (517.12)	3507.34 (452.46)	0.49
Gestational age (mean (SD))	39.50 (1.15)	39.52 (1.13)	0.93
High education of parents (n (%))	44 (80)	44 (71)	0.36
Inhouse smoking (n (%))	2 (3.6)	1 (1.6)	0.92
Breastfeeding, (n (%)) No breastfeeding <3 months 3-6 months >6 months	13 (24) 15 (27) 5 (9) 22 (40)	15 (24) 18 (29) 14 (23) 15 (24)	0.13
Siblings <5y (n (%))	42 (76)	25 (40)	<0.001
Antibiotics for respiratory tract infection in first year of life (n (%))	16 (29)	15 (24)	0.55
RTI episode, at least one (n (%))	21 (38)	21 (34)	0.77

Data was obtained by questionnaires at all routine and RTI visits. Breastfeeding was nonexclusive. High educational level was defined when one of the parents went to university of applied sciences or research university. P-values were determined by chi-square tests or t-test.
* including participants without rhinovirus detection during first year of life

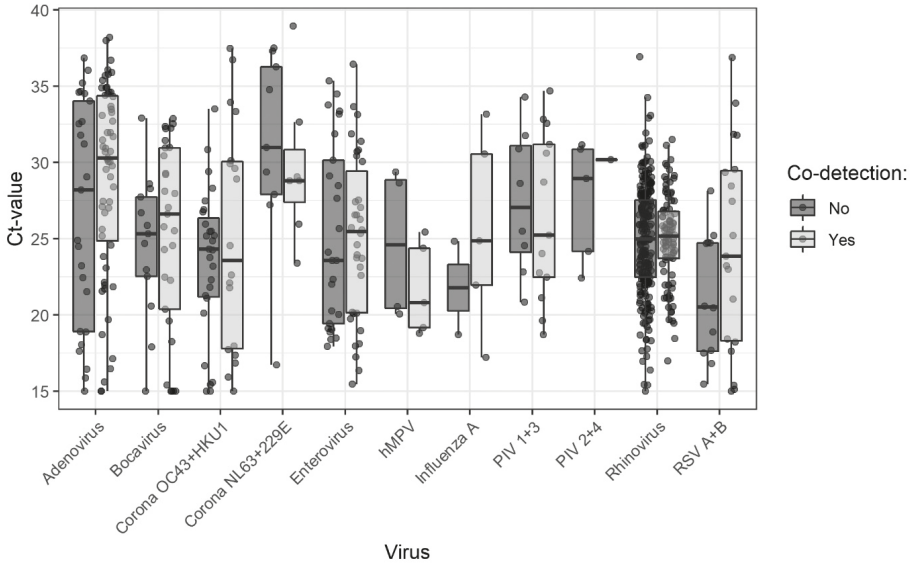
Table, Supplemental Digital Content 12. Mean Ct-value for positive cases per virus and stratified per type of visit.

	Routine visit (n = 429)	RTI visit (n = 51)	p-value
Influenza A (mean (SD))	26.42 (5.63) *(n = 5)*	19.59 (3.34) *(n = 2)*	0.18
RSV A+B (mean (SD))	24.98 (6.34) *(n = 18)*	20.81 (4.74) *(n = 13)*	0.06
HCoV [all] (mean (SD))	26.41 (6.08) *(n = 53)*	21.40 (8.28) *(n = 7)*	0.05*
- HCoV NL63 (mean (SD))	30.58 (4.36) *(n = 13)*	33.99 (6.98) (n = 2)	0.36
- HCoV OC43 (mean (SD))	25.00 (5.74) *(n = 27)*	16.85 (NA) *(n = 1)*	NA
- HCoV 229E (mean (SD))	27.08 (10.28) *(n = 3)*	NA (NA) *(n = 0)*	NA
- HCoV HKU1 (mean (SD))	25.61 (6.08) *(n = 12)*	17.59 (2.70) *(n = 5)*	0.01
PIV [all] (mean (SD))	28.36 (3.99) *(n = 22)*	21.39 (1.16) *(n = 5)*	0.001
- PIV 1+3 (mean (SD))	28.32 (4.45) *(n = 17)*	20.57 (1.79) *(n = 4)*	0.003
- PIV 2+4 (mean (SD))	29.06 (2.86) *(n = 5)*	22.42 (NA) *(n = 1)*	NA
hMPV (mean (SD))	24.72 (4.97) *(n = 4)*	21.67 (3.04) *(n = 5)*	0.29
Adenovirus (mean (SD))	29.08 (5.98) *(n = 67)*	25.13 (8.42) *(n = 17)*	0.03
Bocavirus (mean (SD))	26.04 (5.45) *(n = 32)*	21.85 (5.82) *(n = 10)*	0.04
RV (mean (SD))	24.90 (3.50) *(n = 306)*	26.17 (1.55) *(n = 13)*	0.20
Enterovirus (mean (SD))	25.99 (5.42) *(n = 49)*	20.90 (4.81) *(n = 10)*	0.008
Mean viral load of all viruses (mean (SD))	25.63 (4.20) *(n = 556)*	22.69 (4.97) *(n = 82)*	<0.001

Viral load indicated by Ct-values. P values were determined by t-test.
HCoV: human coronavirus; hMPV: human metapneumovirus; PIV: parainfluenza virus; RSV: respiratory syncytial virus; RV: rhinovirus.
* = non-signifcant (p=0.054)



Figure, Supplemental Digital Content 13. Ct-value per timepoint.
Ct-values of all viruses detected in samples from routine and RTI visits (n=480). Boxplots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line). pp = post-partum (swab taken within 2 hours after birth).



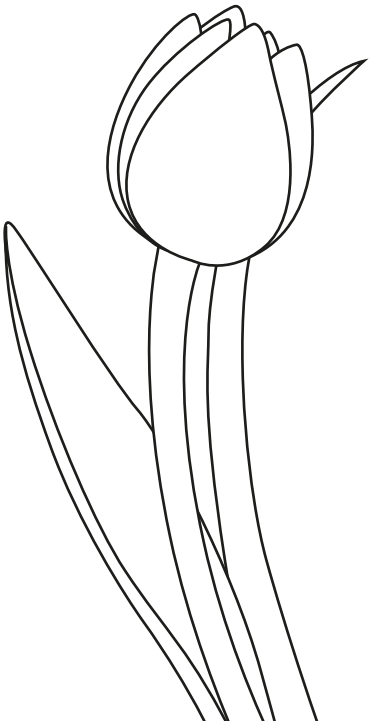
Figure, Supplemental Digital Content 14. Ct-value per virus, grouped by co-detection.
Ct-values of all viruses detected in samples, grouped by whether they were a sole detection or co-detected (n=638). Boxplots represent the 25th and 75th percentiles (lower and upper boundaries of boxes, respectively), the median (middle horizontal line).

Table, Supplemental Digital Content 15. Associations between HCoV detection and RTI.

Variable	aOR	95% CI interval
HCoV HKU1	4.53*	1.49 – 13.73
HCoV NL63 and 229E	1.81	0.32 – 10.20
HCoV OC43	0.36	0.05 – 2.71
Co-detection of other viruses	3.30*	1.64 – 6.63
Age (month)	1.16*	1.10 – 1.23

Odds ratios for associations between HCoV and RTI, in a subgroup of only HCoV detections (n=61), corrected for HCoV type, viral co-detection, and age. Odds ratios (OR) and 95% confidence interval were determined by multivariate generalized estimating equations.
HCoV: human coronavirus

There's gotta be a record
of you someplace
You gotta be on somebody's books
(Dire Straits – On Every Street)



Chapter 5

Respiratory Syncytial Virus Consortium in Europe (RESCEU) Birth Cohort Study: Defining the Burden of Infant Respiratory Syncytial Virus Disease in Europe

Joanne G. Wildenbeest, Roy P. Zuurbier, Koos Korsten, Marlies A. van Houten, Marie N. Billard, Nicole Derksen-Lazet, Matthew D. Snape, Simon B. Drysdale, Hannah Robinson, Andrew J. Pollard, Terho Heikkinen, Steve Cunningham, Amanda Leach, Federico Martinón-Torres, Carmen Rodríguez-Tenreiro Sánchez, Alberto Gómez-Carballa, and Louis J. Bont; for the RESCEU Investigators

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ABSTRACT

Background. Respiratory syncytial virus (RSV) causes significant morbidity and mortality in infants worldwide. Although prematurity and cardiopulmonary disease are risk factors for severe disease, the majority of infants hospitalized with RSV are previously healthy. Various vaccines and therapeutics are under development and expected to be available in the near future. To inform the use of these new vaccines and therapeutics, it is necessary to determine the burden of RSV disease in Europe. We will prospectively follow-up a birth cohort to obtain incidence data on RSV acute respiratory tract infection (ARTI).

Methods. Multicenter prospective study of a birth cohort consisting of 10 000 healthy infants, recruited during 3 consecutive years. RSV associated hospitalization in the first year of life will be determined by questionnaires and hospital chart reviews. A nested cohort of 1000 infants will be actively followed. In case of ARTI, a respiratory sample will be collected for RSV molecular diagnosis.

Results. The primary outcome is the incidence rate of RSV-associated hospitalization in the first year of life. In the active cohort the primary outcome is RSV associated ARTI and MA-ARTI.

Conclusions. We will provide key information to fill the gaps in knowledge about the burden of RSV disease in healthy infants.

Clinical Trials Registration. NCT03627572.

Keywords

respiratory syncytial virus; infant; birth cohort; disease severity; hospitalization; Europe.

BACKGROUND

Human respiratory syncytial virus (RSV) causes severe disease in individuals at the extremes of the age spectrum and in high-risk groups. It was estimated that in 2015, RSV was associated with 33.1 million acute lower respiratory tract infections, 3.2 million RSV-related hospital admissions, and an overall mortality of 118 200 in children < 5 years of age worldwide [1]. These estimates were based on few data and there is a substantial gap in knowledge on morbidity and associated healthcare and societal costs in Europe. Although prematurity and cardiorespiratory comorbidity are well-known risk factors for severe disease in young children, the majority of children admitted to pediatric intensive care units because of severe RSV acute respiratory tract infections (ARTIs) are previously healthy infants [2–4]. Data about RSV incidence and burden of disease in healthy children are scarce, since most studies are performed only in high-risk groups. Moreover, RSV infection in childhood is associated with subsequent wheezing and asthma [5–7], and these long-term sequelae pose a substantial additional burden on the healthcare system.

Treatment and prophylaxis options are limited. Ribavirin has been used as treatment but is not routinely recommended in light of limited evidence of benefit [8]; hence, only supportive care is available for infants with severe RSV infection. Passive prophylaxis with RSV-specific antibodies (palivizumab) is only available for high-risk groups (prematurely born infants and infants with significant cardiac and/or respiratory comorbidity).

Various new RSV vaccines and therapeutics are expected to be available in the near future [9]. To properly evaluate the implementation of these new vaccines and therapeutics, it is necessary to determine the burden of RSV disease in Europe to gain better insight into disease severity in young children and the associated societal and healthcare costs. One way to obtain more detailed data about burden of RSV disease and associated socioeconomic impact is by means of a prospective birth cohort study. Only a few prospective birth cohort studies were performed to evaluate the burden of RSV infection in healthy infants [10–12]. These studies were all relatively small single-center studies with <1000 participants (range, 298–923). Two studies were performed in Europe [10, 12], while 1 study was performed in Kenya [11]. There is a parallel need to assemble clinical resources to identify the correlates of severe

RSV disease for clinical management, classification of disease severity in clinical trials, and identification of biomarkers for severe disease, which are currently lacking [13].

For this purpose, the Respiratory Syncytial Virus Consortium in Europe (RESCEU) has been established. RESCEU will perform the largest prospective multicenter study in healthy children to provide accurate data on RSV disease incidence and sequelae (long-term airway morbidity, including asthma) and economic consequences of RSV infection. We will prospectively follow up a birth cohort of 10 000 healthy children during at least 1 year to obtain incidence data on RSV ARTI, medically attended RSV ARTI, and hospitalization due to RSV

METHODS

Objectives

The primary objective of the RESCEU birth cohort study is to determine the incidence of RSV infection–associated ARTI, RSV-associated medically attended ARTI, and RSV-related hospitalization during the first year of life (Figure 1).

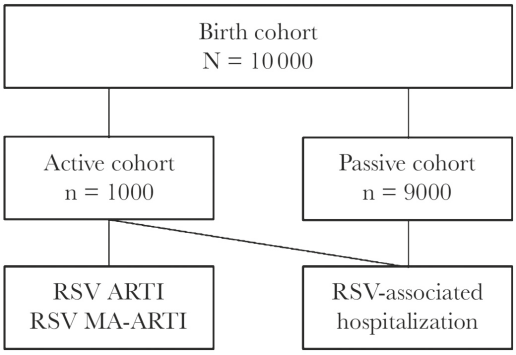

Figure 1. Visual representation of study cohorts and endpoints. Abbreviations: ARTI, acute respiratory tract infection; MA, medically attended; RSV, respiratory syncytial virus.

In addition, the following secondary objectives will be assessed:

1. To estimate how RSV infection of different severity relates to wheeze up to 3 years of age.
2. To determine the rate of all-cause medically attended (inpatient or outpatient) ARTI.

3. To determine mortality (RSV-associated and all-cause) through all RSV seasons of follow-up.
4. To determine healthcare costs, healthcare resource use, interruption of normal activities, and health-related quality of life in RSV-associated and all-cause medically attended (inpatient or outpatient) ARTI patients and their families.
5. To determine the incidence of RSV-related secondary bacterial respiratory tract infections, defined as doctor's diagnosis of a bacterial respiratory tract infection, and antibiotic use within 21 days after onset of RSV infection in hospitalized RSV ARTI patients and nonhospitalized RSV ARTI patients.
6. To collect clinical samples for biomarker analysis from a subset of infants in the active cohort.
7. To determine the incidence rate of other respiratory pathogens associated with all medically attended (inpatient or outpatient) ARTI.
8. To determine the proportion of viral ARTI attributable to RSV.
9. To determine important risk factors for RSV infection (by severity and healthcare utilization).

Study Design

This is a multicountry, multicenter, prospective, observational cohort study.

Study Period

Continuous recruitment will take place between July 2017 and December 2019 (active cohort) or April 2020 (all) to create a cohort with evenly distributed dates of birth over the year and to include several RSV seasons. All participants will be followed up at least to the age of 1 year. Participants of the active cohort will be actively followed up during the 2017–2018, 2018–2019, and 2019–2020 RSV seasons.

Study Population

The birth cohort will consist of 10 000 healthy infants, recruited from the general population. Infants are recruited from maternity wards during the first days after birth in the following 5 participating centers: Spaarne Gasthuis, Haarlem, the Netherlands; Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain; participating hospitals in the Thames Valley and South Midlands Clinical Research Network (Oxford), United Kingdom; Royal Hospital for Sick Children, Edinburgh, United Kingdom; and Turku University Hospital, Turku, Finland.

Inclusion Criteria

Included will be healthy children with a gestational age of at least 37 weeks + 0 days, born in the catchment area of participating centers. Children with perinatal problems, including mild to moderate asphyxia, respiratory distress, or suspected early-onset neonatal infection will be included and are distinguished and analyzed separately at end of study. Parents or legal guardians will be able to communicate in the local language.

Exclusion Criteria

Children with a clinically significant medical illness including cardiovascular, respiratory, renal, gastrointestinal, hematological, neurological, endocrine, immunological, musculoskeletal, oncological, or congenital disorders will be excluded from study participation. Any acute severe medical condition present at the time of sampling in the first week of life (eg, sepsis, severe asphyxia, for which the child is admitted to the hospital) is defined as an exclusion criterion for participation in the active birth cohort. Other exclusion criteria are receipt of maternal RSV vaccine during pregnancy and being in social care.

Recruitment and Informed Consent Procedure

Recruitment will take place during the perinatal period by distribution of information letters and direct contact by study investigators. The investigator will explain the nature of the study and will inform the parents/legal guardian(s) of the infant that participation is voluntary and that they can withdraw from the study at any time. Written informed consent will be obtained from parent(s)/legal guardian(s) of each subject prior to any study procedure.

Study Procedures

All Participants

Parents of all infants who provide consent to participate in the study will be asked to fill out a questionnaire at inclusion and at the age of 1 year. The information collected at baseline will contain details about pregnancy, perinatal course, and potential risk factors for RSV hospitalization and long-term wheeze and asthma, including socioeconomic status, maternal/paternal smoking, presence of siblings, and family history of asthma, allergy, and/or eczema. In the first-year questionnaire, parents will be asked, among other questions, if their child was hospitalized because of an ARTI, in which case the study team will review the hospitalization chart for

admission details and the results of RSV testing. All participating centers will perform RSV testing as standard of care in infants hospitalized with ARTI or will monitor admissions for ARTIs to test children from the RESCEU cohort for RSV.

Active Cohort

Within the birth cohort, a nested cohort of 1000 infants will be actively followed up during the first year of life (active cohort). In addition to the baseline and 1 year questionnaire, samples will be collected in the first week of life (Figure 2 and Supplementary Table 1). These infants will be actively followed up during RSV season(s) in their first year of life (1 October to 1 May, or longer if RSV is still circulating) by weekly contact inquiring about respiratory symptoms. An ARTI is defined as the presence of any of the following symptoms for at least 1 day: runny or blocked nose, coughing, wheezing, or dyspnea. In case of ARTI, a member of the study team will visit the infant within 72 hours after notification of the study team and take a nasal swab for RSV polymerase chain reaction (PCR). If the infant develops an ARTI, parents will also be asked to fill out a diary for 14 days about severity of symptoms, quality of life, healthcare usage, and parental absenteeism from work. The adapted parental version of the Respiratory Syncytial Virus Network (ReSViNET) scale will be used to determine symptom severity [14].

Biomarker Substudy

Three of the 5 centers (Spaarne Gasthuis Haarlem/ Hoofddorp, Hospital Clínico Universitario de Santiago, and participating hospitals in the Thames Valley [Oxford region]) are also participating in the biomarker substudy. The aim of the biomarker substudy is to find markers for disease severity of RSV infection. In these centers an RSV point-of-care test (POCT) will be performed directly on the collected nasal swab from infants in the active cohort at the moment of an ARTI. If the RSV POCT is positive, additional samples will be collected at the moment of infection and 6–8 weeks later (Figure 2 and Supplementary Table 1).

Evaluation of Long-term Sequelae

To evaluate long-term sequelae, parents of all infants participating in the active cohort and of all infants hospitalized because of an ARTI will receive a questionnaire at the age of 2 and 3 years about long-term sequelae and quality of life.

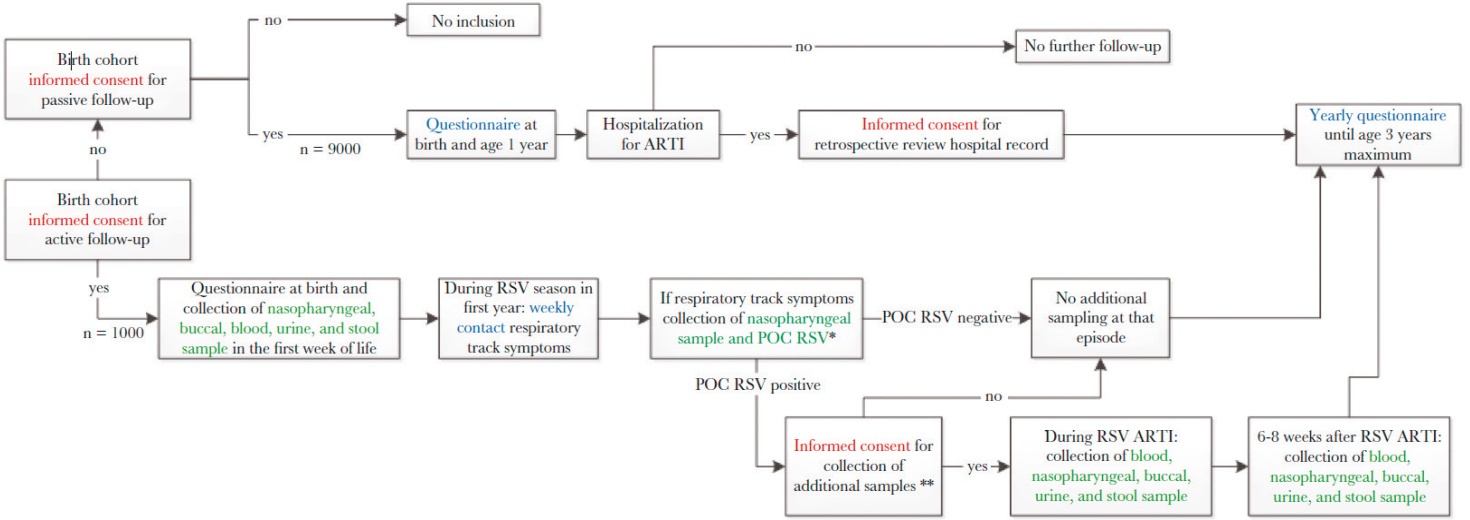

Figure 2. Overview of study design and main procedures of the birth cohort study. *Every episode of respiratory tract symptoms during respiratory syncytial virus (RSV) season (1 year), point-of-care test RSV depending on participating center. **Maximum 1 RSV respiratory tract infection episode per subject (maximum = 480 subjects). Abbreviations: ARTI, acute respiratory tract infection; POC, point of care; RSV, respiratory syncytial virus.

RSV Testing

Sample Collection ARTI (Active Cohort)

All ARTI samples will be collected by a trained member of the study team by taking a nasal flocked swab (FLOQSwab, Copan Diagnostics), which will be directly stored in viral transport medium (MicroTest M4RT, Remel; 3 mL). Collected samples will be tested for RSV by POCT directly and/or stored in aliquots at −80°C.

RSV POCT

RSV POCT will be done at Spaarne Gasthuis Haarlem, Hospital Clínico Universitario de Santiago, and participating hospitals in the Thames Valley [Oxford region]). The Alere i RSV assay (Alere Inc, Waltham, Massachusetts) [15] will be used as the RSV POCT. Staff will get hands-on training on participant sampling and how to perform the Alere i RSV POCT according to the manufacturer's instructions. In short, 200 µL of the viral transport medium mixed with the swab will be aspirated with the included transfer pipette and added to the sample receiver liquid (elution buffer) and mixed for 10 seconds. After initiating the test, results will be displayed within 15 minutes as either RSV positive or negative. The remaining sample will be stored in aliquots at −80°C.

RSV PCR

After active surveillance has finished, RSV quantitative reverse-transcription PCR will be performed on all ARTI samples collected during the study.

Outcomes

The primary outcome is the incidence rate of RSV-associated ARTI leading to hospitalization in the first year of life. In the active cohort, the primary outcome is RSV-associated ARTI and medically attended RSV infection, defined as any medical care for RSV infection.

Secondary Endpoints

1. Parent-reported wheeze and doctor's diagnosis of wheeze by routine care.
2. Incidence of all-cause medically attended (inpatient or outpatient) ARTI.
3. Mortality through all RSV seasons of follow-up including RSV-associated deaths and all-cause deaths.
4. Healthcare utilization for RSV-associated and all-cause medically attended (inpatient or outpatient) ARTI or respiratory events.

5. Incidence of doctor's diagnosed RSV-associated secondary bacterial pneumonia and antibiotic consumption events within 21 days after onset of RSV-related symptoms.
6. Biomarkers of risk, severity of disease, and long-term outcome of RSV infection.
7. Incidence of other respiratory pathogens associated with all medically attended (inpatient or outpatient) ARTI.
8. Proportion of viral infections attributable to RSV.
9. Risk factors for RSV.

Ethical Considerations

The study will be conducted according to the principles of the Declaration of Helsinki (www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act and other relevant guidelines, regulations, and acts. The study was approved by the institutional review board of the University Medical Center Utrecht, the NHS National Research Ethics Service Oxfordshire Committee A (reference number 15/ SC/0335), the South East Scotland Research Ethics Committee (reference number 15/SS/0086), the Ethics Committee of the Hospital District of Southwest Finland, and Hospital Clínico Universitario de Santiago de Compostela. The protocol and patient information have also been reviewed by a member of the RESCEU patient advisory board.

Statistical Methods

Sample Size Calculation

For the primary analysis, the ratio between cases of RSV-related hospitalizations in the first year of life and total number of children in the study will be calculated (full birth cohort). In addition, the ratio between the cases of medically attended RSV ARTI and the number of children undergoing active surveillance will be calculated (active cohort).

For sample size calculation, a yearly incidence of hospitalization of 0.7% was assumed based on previous literature [4, 16]. A sample size of 8700 will produce a 2-sided 95% confidence interval with a half-width of 0.002 (confidence interval formula: exact, Clopper–Pearson). Accounting for a 10% loss to follow-up, 10 000 children will be included in the full birth cohort (Table 1).

Table 1. Expected Incidence of Respiratory Syncytial Virus (RSV) Hospitalization and Medically Attended RSV

Healthy baby, GA at least 37 weeks + 0 days (full cohort)	NL, UK, SP, FI	RSV-related hospitalization	10 000	1	0.7 (4, 16)	0.5–1.3
Healthy baby, GA at least 37 weeks + 0 days (active cohort)	NL, UK, SP, FI	MA RSV	1000	1	10 (4, 16, 17)	8.0–12.0

Abbreviations: CI, confidence interval; FI, Finland; GA, gestational age; MA, medically attended; NL, Netherlands; RSV, respiratory syncytial virus; SP, Spain; UK, United Kingdom.

Statistical Analysis

Descriptive statistics will be used to describe the incidence rate of RSV-associated hospitalization, medically attended RSV ARTI, and non–medically attended RSV ARTI in the birth cohort. Baseline characteristics of the passive and active cohort will be compared. Demographic parameters, clinical parameters and outcome, and laboratory test results will be displayed as categorical data with percentages or as continuous variables with mean (standard deviation) and/or median (interquartile range). Comparisons between groups will be performed using $\chi 2$ test for categorical variables, Student t test for normally distributed continuous variables, or Mann–Whitney U test for not normally distributed continuous variables. Multivariate regression analysis will be performed to analyze multiple risk factors for RSV disease. Statistical analyses will be performed using SPSS version 20 or a more recent version or with R statistical software version 3.5.1 or higher.

Dissemination and Publication

Results of this study will be disclosed unreservedly. Data generated within this study will be made available to other investigators through a secure central information repository. A virtual biobank will be set up for collected samples.

DISCUSSION

Various studies have evaluated the burden of RSV disease in infants. The study by Hall et al showed that RSV causes substantial morbidity in children aged < 5 years in the United States. Disease burden was highest in infants aged < 6 months with an annual hospitalization rate of 1.7%. Most children admitted with RSV were previously healthy [4]. In a recent systematic review, Shi et al [1] collected data from all published and unpublished population-based studies about RSV infection in infants < 5 years

of age over the last 20 years to estimate the total RSV-associated disease burden worldwide. They estimated that worldwide, 1.4 million RSV-related hospitalizations and 27 300 in-hospital deaths occurred in infants aged < 6 months, accounting for 45% of the total number of hospitalizations and deaths in children aged < 5 years [1]. Although they were able to collect substantially more data compared to their previous systematic review [17], the uncertainty range of their estimations remained substantial, leading to the conclusion that more detailed data about RSV disease burden are needed. This is especially important for future introduction of RSV vaccines, because policymakers will need this information to decide whether to introduce these vaccines and to evaluate the effect on morbidity and mortality after introduction. With the current cohort study, we aim to provide accurate data about RSV-related hospitalization as well as the burden of RSV disease in Europe.

The development of an RSV vaccine has been identified as a priority by the World Health Organization (WHO) [15]. To date, > 40 vaccines against RSV are in development, varying from preclinical to phase 3 [18]. Since the main population at risk for severe disease is infants in the first months of life, who are too young to be protected by active immunization, other strategies have been developed [19]. One strategy is maternal vaccination, which aims to protect infants from birth through the first 3–6 months of life by transfer of protective maternal antibodies during the second half of pregnancy. Maternal vaccination against pertussis has already proven that this strategy is very effective in preventing disease in young infants [20]. Results of a recent phase 3 trial of maternal vaccination with a RSV F-protein nanoparticle vaccine (PREPARE trial) showed protection against severe RSV in infants < 90 days of age, but the study did not reach its primary endpoint [21].

Another strategy is to administer monoclonal antibodies against RSV to young infants during the RSV season. To date, palivizumab is the only market-approved monoclonal antibody, but is registered for high-risk infants. Due to the high costs, palivizumab is only affordable for high-risk infants in developed countries. In addition, monthly intramuscular injections are necessary. A promising candidate is Medi8897, an extended half-life monoclonal antibody against RSV F. In a recent phase 2b trial in preterm infants of a gestational age of 29–35 weeks, a 78% reduction in the incidence of RSV-related hospitalization and a 70% reduction in the incidence of medically attended RSV was seen [22]. Because the development of an RSV vaccine has been prioritized not only by the WHO, but also by the United States Food and

Drug Administration and the European Medicines Agency, promising candidates in later stages of development could expect support and accelerated evaluation from these organizations to obtain faster market approval.

With this in mind, expectations are that within 5 years an approved product for prevention of RSV for all infants will be on the market. Subsequently, governments will have to decide whether this new vaccine would be eligible to be implemented into their national immunization schedule. Information about RSV incidence and associated burden on healthcare use as well as economic and societal impact and long-term sequelae in the population is imperative to evaluate the possible benefit of introducing a new vaccine into a national immunization program. With this study, we aim to provide this key information to fill the gaps in knowledge about the burden of RSV disease in healthy infants and help regulators, governments, and other stakeholders with decision making.

NOTES

RESCEU investigators

Joanne Wildenbeest, Roy Zuurbier, Koos Korsten, Marlies van Houten, Marie Billard, Nicole Derksen-Lazet, and Louis Bont (University Medical Center Utrecht); Simon Drysdale, Matthew Snape, Hannah Robinson, and Andrew Pollard (University of Oxford); Federico Martinón-Torres, Carmen Rodríguez-Tenreiro Sánchez, and Alberto Gómez-Carballa (Servicio Galego de Saude); Terho Heikkinen (University of Turku and Turku University Hospital); Steve Cunningham, Harish Nair, and Harry Campbell (University of Edinburgh); Amanda Leach (GlaxoSmithKline); Peter Openshaw (Imperial College London); Philippe Beutels (Universiteit Antwerpen); Eva Molero (Synapse); Adam Meijer and Elisabeth Sanders (Netherlands National Institute for Public Health and the Environment); Thea Kølsen Fischer (Statens Serum Institut); Maarten van den Berge (Academisch Ziekenhuis Groningen); Carlo Giaquinto (PENTA Foundation); Mark Esser (AstraZeneca); Charles Knirsch (Pfizer); Scott Gallichan, (Sanofi Pasteur); Jeroen Aerssens (Janssen); and Brian Rosen (Novavax).

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Disclaimer

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Conflict of interests

L. J. B. has regular interaction with pharmaceutical and other industrial partners, but has not received personal fees or other personal benefits; and is the founding chairman of the Respiratory Syncytial Virus Network (ReSViNET) Foundation. The University Medical Center Utrecht has received major funding (>€100 000 per industrial partner) for investigator-initiated studies from AbbVie, MedImmune, Janssen, the Bill & Melinda Gates Foundation, Nutricia (Danone), and MeMed Diagnostics; has received major cash or in-kind funding as part of the public-private partnership Innovative Medicines Initiative–funded RESCEU project from GSK, Novavax, Janssen, AstraZeneca, Pfizer, and Sanofi; has received major funding by Julius Clinical for participating in the INFORM study sponsored by MedImmune; has received minor funding for participation in trials by Regeneron and Janssen during 2015–2017 (total annual estimate < €20 000); and has received minor funding for consultation and invited lectures by AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, Novavax, Pfizer, and Janssen (total annual estimate < €20 000). F. M-T. has received honoraria from GSK, Pfizer, Sanofi Pasteur, Merck Sharp & Dohme, Seqirus, and Janssen for taking part in advisory boards and expert meetings, and for acting as speaker in congresses outside the scope of the submitted work; has also acted as principal investigator in randomized controlled trials of the above-mentioned companies as well as Ablynx, Regeneron, Roche, Abbott, Novavax, and Medimmune, with honoraria paid to his institution; and his research activities received support from the Instituto de Salud Carlos III (Proyecto de Investigación en Salud, Acción Estratégica en Salud): project ReSVinext ISCIII/PI16/01569/ Cofinanciado FEDER; Consellería

de Sanidade, Xunta de Galicia (RHI07/2-intensificación actividad investigadora, PS09749 and 10PXIB918184PR), Instituto de Salud Carlos III (Intensificación de la actividad investigadora 2007–2012, PI16/01569), Fondo de Investigación Sanitaria (PI070069/ PI1000540) del plan nacional de I+D+I and "fondos FEDER," and 2016-PG071 Consolidación e Estructuración REDES 2016GI-1344 G3VIP (Grupo Gallego de Genética Vacunas Infecciones y Pediatría, ED341D R2016/021. M. D. S. acts on behalf of the University of Oxford as an investigator on studies sponsored and/or funded by vaccine manufacturers including Novavax, GlaxoSmithKline, Janssen, Medimmune, MCM, and Pfizer, but receives no personal financial benefit for this work. A. J. P. is Chair of the DHSC's JCVI and the European Medicines Agency Scientific Advisory Group on Vaccines; is a member of the WHO's Strategic Advisory Group of Experts on Immunization; and is an NIHR Senior Investigator. S. C. provides consultancy with fees paid to the University of Edinburgh for Ablynx (Sanofi), Janssen, Pulmocide, and ReViral; and is a principal investigator for studies relating to respiratory syncytial virus (RSV) therapeutics for MedImmune. S. B. D. acts on behalf of St George's, University of London as an investigator on studies sponsored and/or funded by vaccine manufacturers including Janssen and Medimmune, but receives no personal financial benefit for this work. T. H. has received honoraria from Janssen and Sanofi Pasteur for participation in ad hoc advisory board meetings and independent data monitoring committees. Turku University Hospital (a secondary employer of T. H.) has received a grant from Janssen for RSV epidemiologic studies unrelated to this protocol. A. L. is an employee of GlaxoSmithKline and holds shares in the company. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

REFERENCES

1. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017; 390:946–58.
2. Haerskjold A, Kristensen K, Kamper-Jørgensen M, Nybo Andersen AM, Ravn H, Graff Stensballe L. Risk factors for hospitalization for respiratory syncytial virus infection: a population-based cohort study of Danish children. Pediatr Infect Dis J 2016; 35:61–5.
3. Hall CB, Weinberg GA, Blumkin AK, et al. Respiratory syncytial virus–associated hospitalizations among children less than 24 months of age. Pediatrics 2013; 132:e341–8.
4. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009; 360:588–98.
5. Blanken MO, Rovers MM, Bont L; Dutch RSV Neonatal Network. Respiratory syncytial virus and recurrent wheeze. N Engl J Med 2013; 369:782–3.
6. Lotz MT, Moore ML, Peebles RS Jr. Respiratory syncytial virus and reactive airway disease. Curr Top Microbiol Immunol 2013; 372:105–18.
7. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999; 354:541–5.
8. Ventre K, Randolph AG. Ribavirin for respiratory syncytial virus infection of the lower respiratory tract in infants and young children. Cochrane Database Syst Rev 2007; 24:CD000181.
9. Mazur NI, Martinón-Torres F, Baraldi E, et al; Respiratory Syncytial Virus Network (ReSViNET). Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. Lancet Respir Med 2015; 3:888–900.
10. Houben ML, Bont L, Wilbrink B, et al. Clinical prediction rule for RSV bronchiolitis in healthy newborns: prognostic birth cohort study. Pediatrics 2011; 127:35–41.
11. Nokes DJ, Okiro EA, Ngama M, et al. Respiratory syncytial virus infection and disease in infants and young children observed from birth in Kilifi district, Kenya. Clin Infect Dis 2008; 46:50–7.
12. Toivonen L, Karppinen S, Schuez-Havupalo L, et al. Respiratory syncytial virus infections in children 0-24 months of age in the community. J Infect 2020; 80:69–75.
13. Brown PM, Schneeberger DL, Piedimonte G. Biomarkers of respiratory syncytial virus (RSV) infection: specific neutrophil and cytokine levels provide increased accuracy in predicting disease severity. Paediatr Respir Rev 2015; 16:232–40.
14. Justicia-Grande AJ, Pardo-Seco J, Cebey-López M, et al; Respiratory Syncytial Virus Network (ReSViNET). Development and validation of a new clinical scale for infants with acute respiratory infection: the ReSViNET scale. PLoS One 2016; 11:e0157665.
15. World Health Organization. RSV vaccine research and development. https://www.who.int/immunization/research/development/ppc_rsv_vaccines/en/. Accessed 27 November 2019.
16. Zomer-Kooijker K, Uiterwaal CS, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. Eur Respir J 2014; 44:666–74.
17. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375:1545–55.

18. PATH. RSV vaccine and mAb snapshot. https://path.org/ resources/rsv-vaccine-and-mab-snapshot/. Accessed 27 November 2019.
19. Mazur NI, Higgins D, Nunes MC, et al; Respiratory Syncytial Virus Network (ReSViNET) Foundation. The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. Lancet Infect Dis 2018; 18:e295–311.
20. Amirthalingam G, Andrews N, Campbell H, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. Lancet 2014; 384:1521–8.
21. Madhi S, Swamy GK, Muñoz FM, et al. Phase 3 PREPARE study: efficacy and safety of an RSV vaccine administered to pregnant women. In: 37th Annual Meeting of the European Society for Paediatric Infectious Diseases, Ljubljana, Slovenia, 6–11 May 2019.
22. Griffin MP, Yuan Y, Takas T, et al. MEDI8897 prevents serious RSV disease in healthy preterm infants. Open Forum Infect Dis 2019; 6:S27.

Supplementary table 1. Sample collection in the active cohort and biomarker sub-study.

Moment of sampling	Sample	Volume	Analysis
In the first week after birth (day 5 +/- 2)	Serum (capillary/ venous)	Max 1.8 ml	RSV serology Proteome*
	Paxgene (capillary/venous)	0.2- 0.5 ml	Transcriptome*
	Whole blood (only if venous)	Max 1 ml	Cellular immunology*
	Nasopharyngeal swab	n/a	Airway microbiome Airway transcriptome
	Buccal swab	n/a	DNA/GWAS
	Stool	5-10 ml (min 2 ml)	Microbiome
	Urine	3 ml	Metabolomics
ARTI	Nasal swab	n/a	RSV POCT (qualitative) RSV RT-PCR# (quantitative)
Biomarker substudy: RSV ARTI and convalescence	Serum (venous)	1-2 ml	RSV serology Proteome*

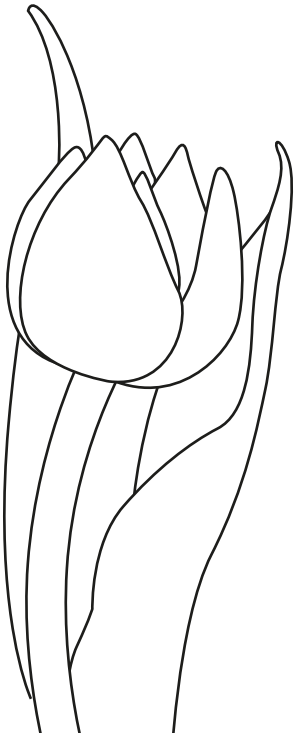
(6-8 weeks later)		
Paxgene (venous)	0.2-0.5 ml	Transcriptome*
Whole blood (venous)	1-2 ml	Cellular immunology*
Stool	5-10 ml (min 2 ml)	Microbiome
Nasopharyngeal swab		Airway microbiome Airway transcriptome
Urine	3 ml	Metabolomics
Buccal swab	n/a	DNA/GWAS

* and additional RSV-related biomarkers

and multiplex RT-PCR respiratory viruses in case of RSV ARTI

Abbreviations: RSV, respiratory syncytial virus; n/a, not applicable; DNA, deoxyribonucleic acid; GWAS, genome-wide association study; ARTI, acute respiratory tract infection; POCT, Point of care test; RT-PCR, reverse transcription polymerase chain reaction

You don't need to be coy, Roy
(Paul Simon – 50 Ways to Leave Your Lover)



Chapter 6

The burden of RSV in healthy term-born infants in Europe: a prospective birth cohort study

Joanne G. Wildenbeest, Roy P. Zuurbier *, Marie-Noëlle Billard*, Koos Korsten, Annefleur C. Langedijk, Peter M. van de Ven, Matthew D. Snape, Simon B. Drysdale, Andrew J. Pollard, Hannah Robinson, Terho Heikkinen, Steve Cunningham, Thomas O'Neill, Bishoy Rizkalla, Ana Dacosta-Urbieta, Federico Martinón-Torres, Marlies A. van Houten, Louis J. Bont, for the RESCEU Investigators
* These authors contributed equally

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ABSTRACT

Background. Respiratory syncytial virus (RSV) is a major cause of hospitalisation in infants. The burden of RSV infection in healthy term infants has not yet been established. Accurate health-care burden data in healthy infants are necessary to determine RSV immunisation policy when RSV immunisation becomes available.

Methods. We performed a multicentre, prospective, observational birth cohort study in healthy term-born infants (≥37 weeks of gestation) in five sites located in different European countries to determine the health-care burden of RSV. The incidence of RSV-associated hospitalisations in the first year of life was determined by parental questionnaires and hospital chart reviews. We performed active RSV surveillance in a nested cohort to determine the incidence of medically attended RSV infections. The study is registered with ClinicalTrials.gov, NCT03627572.

Findings. In total, 9154 infants born between July 1, 2017, and April 1, 2020, were followed up during the first year of life and 993 participated in the nested active surveillance cohort. The incidence of RSV-associated hospitalisations in the total cohort was 1·8% (95% CI 1·6–2·1). There were eight paediatric intensive care unit admissions, corresponding to 5·5% of 145 RSV-associated hospitalisations and 0·09% of the total cohort. Incidence of RSV infection in the active surveillance cohort confirmed by any diagnostic assay was 26·2% (24·0–28·6) and that of medically attended RSV infection was 14·1% (12·3–16·0).

Interpretation. RSV-associated acute respiratory infection causes substantial morbidity, leading to the hospitalisation of one in every 56 healthy term-born infants in high-income settings. Immunisation of pregnant women or healthy term-born infants during their first winter season could have a major effect on the health-care burden caused by RSV infections.

Funding. Innovative Medicines Initiative 2 Joint Undertaking, with support from the EU's Horizon 2020 research and innovation programme and European Federation of Pharmaceutical Industries and Associations.

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed, using the terms "RSV" or "respiratory syncytial virus", "hospitalisations", and "infant" or "first year of life", on May 31, 2022, for studies published between Jan 1, 1993, and May 31, 2022, with no language restrictions. The results, 4957 articles, included mostly retrospective analyses of RSV-coded hospitalisations from health registries or prospective studies conducted in a single country. These studies emphasised the large morbidity and mortality burden in young children associated with RSV. In a systematic review and meta-analysis from The Lancet, RSV was estimated to be associated with 3·6 million hospitalisations for acute lower respiratory infections and 101 400 in-hospital or out-of-hospital deaths in children younger than 5 years, annually, worldwide. A gap exists in the knowledge of the RSV burden in healthy term infants, the largest population of RSV-infected infants. We identified ten birth cohort studies that reported RSV-associated hospitalisation in infants with estimates varying between 0·6% and 5%. These birth cohorts had relatively small sample sizes with 156 to 1143 participants, and only five included only healthy term-born children. The reliability and the precision of these estimates can be improved by large prospective birth cohorts conducted in multiple countries. Several maternal vaccines and passive immunisation against RSV are currently at advanced stages of clinical development or under review for licensure. To decide how these new prevention strategies should be included in national vaccination programmes, precise estimates of the health-care burden of RSV infections in the first months of life are required.

Added value of this study

The RESCEU birth cohort study is the largest multicentre prospective birth cohort that evaluated the incidence of RSV-associated hospitalisations and medically attended acute respiratory infections. It was designed to provide a precise and up-to-date estimate of the total RSV incidence and health-care burden in Europe. Almost 10 000 participants were enrolled in five European countries and 97% were successfully followed up during the first year of life. To estimate the incidence of medically attended RSV infections, we actively followed up a nested cohort of approximately 1000 participants.

The incidence of RSV-confirmed hospitalisations in the first year of life was 1·8% (95% CI 1·6–2·1). About half of hospitalisations for respiratory tract infection in the

first year of life were associated with RSV. The majority (57·9%) of RSV-associated hospitalisations occurred in children younger than 3 months. The incidence of medically attended RSV infections was 14·1% (12·3–16·0).

Implications of all the available evidence

This study provides the precise estimates of the health-care burden of RSV required to decide on future RSV immunisation programmes. The health-care burden of RSV among healthy infants is considerable in Europe, with one in 56 healthy term-born infants hospitalised for RSV infection annually. As the incidence of severe RSV infection is highest in the first months of life, maternal vaccination as well as passive infant immunisation could have a major effect on the health of healthy term infants.

INTRODUCTION

Respiratory syncytial virus (RSV) causes a substantial burden of disease in infants worldwide with an estimated annual mortality of 101 400 in children younger than 5 years [1]. Although more than 97% of RSV-attributable deaths occur in low-income and middle-income countries, the health-care burden of RSV infection in high-income countries is considerable, with an estimated annual hospitalisation rate of three per 1000 children younger than 5 years in the USA [2]. Passive immunisation against RSV with palivizumab is available for high-risk groups, including premature infants and children with congenital heart disease or bronchopulmonary dysplasia. Because the majority of children hospitalised with RSV have no pre-existing conditions, a high morbidity is seen in infants younger than 6 months despite the availability of palivizumab [2]. Various maternal vaccine and passive immunisation trials, which aim to protect all infants in the first months of life, are currently in phase 3 or submitted for regulatory approval [3–5]. Expectations are that within 1–3 years one or several of these products will be approvedby regulatory authorities and governments will have to decide whether these newly available prevention strategies should be implemented into their national immunisation schedule [6]. Accurate information about RSV health-care burden in healthy infants is essential for decision makers to evaluate the health and economic benefit of these new prevention strategies.

Most large studies that aimed to determine RSV-associated hospitalisation rates in young children included children with comorbidities, were country-specific, and

partly based on estimates instead of actual numbers [2,7,8]. Birth cohort studies estimate disease incidence more accurately, but previous prospective birth cohorts in healthy infants were relatively small (158–1143 participants) and done in one centre or country, restricting generalisability [9–18]. To our knowledge, the largest prospective birth cohort determining RSV burden was a South African, single-centre study that reported 54 RSV-associated hospitalisations in 1143 children (17% with comorbidity) in the first 2 years of life [13]. To prepare for the introduction of RSV immunisation, the Respiratory Syncytial virus Consortium in Europe (RESCEU) international consortium was funded by the EU Commission to obtain accurate data on the incidence and long-term consequences of RSV infection in healthy term infants.

The primary objective of this study was to determine the incidence of medically attended and hospitalised RSV-associated respiratory infections in healthy term infants in Europe. Secondary objectives included estimating the incidence of symptomatic RSV infections, the incidence of all-cause respiratory infections, and the proportion of respiratory infections attributable to RSV.

METHODS

Study design

The study design and protocol have been described previously [19]. In short, healthy term-born infants were enrolled at birth between July 1, 2017, and July 31, 2020, in five sites each located in a different European country representing western, northern, and southern Europe (Spain, Finland, England, Scotland, and the Netherlands). Children born at 37 weeks or more of gestation with no evidence of significant cardiovascular, respiratory, renal, gastrointestinal, haematological, neurological, endocrine, immunological, musculoskeletal, oncological, or congenital disorders were considered healthy term-born [18]. All participating children were followed up for at least 1 year. Children diagnosed with comorbidities later were not systematically excluded. We used parental questionnaires to screen for hospitalisation for acute respiratory infection (ARI) during the first year of life at the age of 1 year. Hospital records, including RSV testing results, were retrospectively assessed in case of hospitalisation for ARI. All participating hospitals tested for RSV during the RSV season as part of standard care and were situated in a distinct geographical area to ensure that children were preferentially referred to that hospital if inpatient care was needed. For infants whose parents did not complete the 1-year questionnaire,

hospital records were screened for ARI hospitalisations within the first year of life in participating hospitals.

At enrolment at all five sites, participants of the birth cohort were also invited to participate in a nested cohort (referred to as active surveillance cohort). Participants of the birth cohort and the active surveillance cohort were recruited on a voluntary basis and therefore were a convenience sample of term-born children living in the catchment area of the sites. To obtain a cohort with evenly distributed months and years of birth over the recruitment period, sites were instructed to recruit 15–20 participants per week, including two participants in the active surveillance cohort. Enrolment in the active surveillance cohort continued until the planned sample size was reached in each site (200 per site). Infants were actively followed up until their first birthday during the RSV seasons of 2017–18, 2018–19, and 2019–20. Between Oct 1 and May 1 (or longer if RSV was still circulating), parents were contacted weekly to report ARI symptoms of their child. In case of an ARI, a study visit was planned within 72 h of notification to obtain a nasal swab for RSV testing. Parents completed a diary with respiratory symptoms and health-care usage for 14 days after symptoms onset [18]. Written or electronic informed consent was obtained from the parents of all study participants.

RSV detection in active surveillance cohort

At all sites, a nasal sample was collected during each ARI episode by using minitip flocked swabs (FLOQSwab, Copan Diagnostics, California, USA), and directly stored in viral transport medium (MicroTest M4RT [Remel, 3 mL]). All samples were stored at –80°C. After the end of the study, all samples were tested with in-house RSV quantitative reverse transcription PCR (RT-qPCR; appendix) [20,21]. In addition, a point of care test (POCT, Alere i RSV assay [Alere, Waltham, MA, USA]) was performed at the time of sample collection at the three sites in Spain, England, and the Netherlands. If the infant had an RSV-positive ARI episode, POCT was not performed during further ARIs. An RSV-positive ARI episode was defined as a positive test result from either in-house RT-qPCR or POCT, or both.

Outcome definitions and statistical analysis

An ARI episode was defined as the onset or worsening of any of the following symptoms for at least 1 day: runny or blocked nose, coughing, wheezing, or dyspnoea [19]. Episodes were associated with RSV if a POCT or in-house PCR test

was positive for RSV. Samples taken more than 10 days after onset were excluded from analysis. Medically attended ARI were defined as ARI episodes with at least one visit to a health-care provider (outpatient clinics, emergency department visits, general practitioner visits) or hospitalisation. RSV-associated hospitalisations, RSV-associated ARI, and medically attended RSV-associated ARI were reported as incidence (ie, the proportion of infants experiencing the event at least once during their first year of life) and as incidence rate per 1000 infant-months (number of events per 1000 infant-months of follow-up). The use of incidence rates in addition to incidence was pre-defined in the statistical analysis plan to account for possible variation in follow-up time due to early dropouts of participants and for participants experiencing outcomes more than once (appendix). Wheezing during the first year of life was defined as at least one wheezing episode reported by parents in the 1-year questionnaire.

Statistical analyses were performed according to the predefined statistical analysis plan (appendix). For sample size calculation of the total cohort, a yearly incidence of hospitalisations of 0·7% was assumed on the basis of previous literature [2,22]. A sample size of 8700 would produce a two-sided 95% Clopper-Pearson CI with a half-width of 0·2% for this incidence. If accounting for 10% loss to follow-up 10 000 infants were to be included.19 Similarly, a sample size of 1000 infants was estimated for the active surveillance cohort, which would produce a two-sided 95% Clopper-Pearson CI with a half-width of 2%, for an assumed incidence of medically attended ARI of 10% [2,9,22]. Baseline characteristics and clinical parameters were summarised by frequency and percentage for categorical variables and mean (SD) or median (IQR) for continuous variables. Baseline characteristics were compared between groups using χ^2 tests for categorical variables, Student's t tests for normally distributed continuous variables and Mann-Whitney U tests for not normally distributed continuous variables. RSV status was assumed negative when hospitalisation occurred outside of the RSV season. RSV status of hospitalisations during the RSV season and ARI in the active surveillance cohort with invalid or missing RSV test results were imputed using multiple imputation based on site, sex, age, and meteorological season at time of hospitalisation or ARI. Any missing observations for medical attendance of ARIs was subsequently imputed using the same set of predictors to which RSV status was added. Imputation yielded ten complete datasets for each of the two cohorts. After imputation, pooled 95% Wilson-score CIs were calculated for the proportion of infants with at least one RSV-associated hospitalisation or ARI in the

first year. Incidence rates were calculated together with 95% CIs based on a Poisson distribution and compared between subgroups of infants using Poisson generalised linear models. Statistical analyses were performed using SPSS (version 26) and R statistical software (version 3.5.1).

The study was approved by the Institutional Review Board of the University Medical Center Utrecht (ref 17/069), National Health Service National Research Ethics Service Oxfordshire Committee A (ref 17/SC/0335) and South East Scotland Research Ethics Committee (ref 17/SS/0086), the Ethics Committee of the Hospital District of Southwest Finland (ref 17201), and Hospital Clínico Universitario de Santiago de Compostela (ref 2017/175).

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies (appendix). The study is registered with ClinicalTrials.gov, NCT03627572.

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report or the decision to submit for publication.

RESULTS

Between July 1, 2017, and July 31, 2020, 9466 healthy term infants were recruited at birth, of whom 9154 (96·7%) were included in the primary analysis (figure 1). Because of the COVID-19 pandemic, 223 infants born after April 1, 2020, were excluded as RSV was not circulating during their first year of life. Between Sept 1, 2017, and Nov 30, 2019, 1041 infants were enrolled in the active surveillance cohort and 993 (95·4%) who participated for at least 4 weeks were included in the analysis (figure 1). Five deaths occurred in study participants, none was related to RSV. There was substantial and expected variation in baseline characteristics between countries (table 1). Non-exhaustively, the most common ethnic origin was according to country geographical location, smokers in the family were more common in Spain, and maternal vaccination was almost never reported in the Netherlands where it was not recommended at the time. Compared with the rest of the cohort, participants of the active surveillance cohort more frequently reported maternal vaccination

against influenza or pertussis, multiple births, a family history of atopy, and parental university level of education, whereas parental smoking and parental origin from northwest Europe were reported less frequently; they also had fewer siblings and were born later in the year than other participants.

We observed 388 ARI hospitalisations (figure 1 and 2, appendix). Of these, 145 (37·4%) were positive for RSV, 193 (49·7%) were negative or occurred outside the RSV season, and 50 (12·9%) occurred during the RSV season but were not tested for RSV (and status was imputed). Among 145 RSV-associated hospitalisations, RSV was detected during admission by hospital laboratory PCR tests in 71 (49·0%) and by POCT in 67 (46·2%). The test used was not documented for seven RSV-associated hospitalisations. Overall, 143 (1·6%) children were hospitalised with confirmed RSV, including two who were admitted twice with RSV. After imputing missing RSV test results, the incidence of RSV-associated hospitalisation was 1·8% (95% CI 1·6–2·1), corresponding to an RSV-associated hospitalisation incidence rate of 1·6 per 1000 infant-months (1·3–1·8; table 2). RSV-associated hospitalisation incidence in countries varied between 1·1% (0·7–1·5) in Finland and 2·5% (1·8–3·4) in Spain (table 3). RSV-associated hospitalisation incidence rate was higher in children born in autumn (2·6 per 1000 infant-months, 2·0–3·3) than in children born in winter (1·1 per 1000 infant-months, 0·8–1·6, Bonferroni adjusted p=0·002) and spring (0·8 per 1000 infant-months, 0·5–1·3, Bonferroni adjusted p=0·001; table 3, appendix). RSV-associated hospitalisation incidence rate was highest in 2017–18 (2·7 per 1000 infant-months, 1·9–4·0) when the proportion of participating children younger than 6 months was high, and lowest in 2019–20 (1·5 per 1000 infant-months, 1·1–1·8; table 3).

Table 1. Baseline characteristics of participants by recruitment sites based on participants with available information.

	Total Cohort						Active surveillance cohort					
Site#	**SCO**	**ENG**	**ESP**	**FIN**	**NLD**	**All**	**SCO**	**ENG**	**ESP**	**FIN**	**NLD**	**All**
Total number of participants	n=2130	n=1979	n=1080	n=2093	n=1879	n=9154	n=203	n=198	n=205	n=200	n=187	n=993
Follow-up time (infant-months)	25,498	23,458	12,949	25,119	22,484	109,507	2,408	2,288	2,404	2,384	2,245	11,728
Pregnancy												
Vaccination (n (%))*	85%	91%	61%	45%	34%	64%	93%	93%	59%	65%	31%	69%
Influenza	68%	73%	28%	45%	1%	46%	76%	72%	19%	65%	3%	47%
Pertussis	82%	86%	58%	0%	34%	51%	89%	91%	57%	1%	30%	54%
Smoking during pregnancy (n (%))	7%	5%	10%	5%	4%	6%	4%	5%	9%	7%	2%	5%
Birth												
Month of birth (n (%))*												
Oct - Dec	24%	22%	26%	21%	28%	24%	15%	13%	34%	19%	33%	23%
Jan - Mar	31%	29%	24%	15%	33%	26%	16%	14%	16%	29%	34%	22%
Apr - Jun	22%	28%	15%	29%	16%	23%	34%	30%	14%	34%	16%	26%
Jul - Sept	23%	22%	36%	34%	23%	27%	35%	42%	36%	18%	18%	30%
Male sex (n (%))	52%	53%	51%	52%	50%	52%	52%	55%	52%	53%	45%	52%
Multiple birth (n (%))*	2%	3%	3%	1%	1%	2%	9%	3%	3%	1%	3%	4%
Cesarean delivery (n (%))*	44%	38%	22%	14%	22%	29%	41%	38%	32%	14%	24%	30%
Birth weight <2500g (n (%))	2%	3%	3%	1%	1%	2%	2%	3%	4%	2%	2%	3%
Antibiotics <72h post-partum	0%	7%	1%	5%	2%	3%	0%	7%	0%	4%	1%	2%
Intention to breastfeed (n (%))*	79%	84%	68%	97%	73%	83%	90%	92%	71%	98%	82%	97%
Family												
Any siblings (n (%))	43%	50%	52%	53%	48%	49%	51%	45%	48%	48%	63%	51%
Number of siblings {Median (IQR))*	1 (1-2)	1 (1-2)	1 (1-1)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-2)	1 (1-1)
Sibling(s) in daycare or primary school	38%	42%	45%	41%	45%	41%	45%	35%	42%	35%	57%	43%
Smokers in the family*	15%	14%	28%	13%	16%	16%	7%	10%	28%	12%	11%	14%
Mother	4%	2%	6%	2%	3%	3%	2%	1%	4%	2%	1%	2%
Father	12%	11%	24%	11%	14%	14%	6%	8%	25%	11%	10%	12%

Table 1. Continued.

	Total Cohort						Active surveillance cohort					
Other family member	1%	2%	3%	0%	1%	1%	0%	2%	3%	0%	1%	1%
Smoking in the house	1%	1%	4%	0%	0%	1%	0%	2%	3%	0%	1%	1%
Family history of atopy*	74%	72%	56%	63%	71%	68%	80%	76%	60%	67%	76%	72%
Sibling(s) uses or used respiratory medicine	8%	11%	11%	8%	11%	9%	5%	8%	8%	5%	15%	8%
Ethnic origin of the mother*												
Northwest Europe	77%	75%	3%	97%	78%	73%	72%	72%	4%	98%	88%	66%
Southern Europe	4%	2%	90%	0%	2%	12%	5%	3%	87%	0%	2%	20%
Other	19%	23%	10%	3%	24%	16%	23%	25%	8%	3%	11%	14%
Ethnic origin of the father*												
Northwest Europe	78%	76%	3%	95%	77%	73%	76%	79%	4%	97%	89%	68%
Southern Europe	4%	3%	90%	1%	1%	12%	3%	2%	88%	0%	1%	29%
Other	18%	23%	9%	5%	24%	16%	20%	20%	7%	4%	11%	12%
Highest level of education of the mother*												
Secondary / vocational school	37%	38%	51%	35%	32%	37%	18%	20%	50%	31%	25%	29%
University of (applied) sciences	63%	62%	45%	63%	67%	61%	82%	80%	46%	67%	75%	70%
Highest level of education of the father*												
Secondary / vocational school	48%	48%	66%	48%	40%	48%	29%	34%	68%	46%	37%	43%
University of (applied) sciences	52%	52%	24%	48%	58%	49%	71%	65%	27%	51%	63%	55%
Employment of the mother before birth												
Full-time	65%	64%	59%	69%	42%	60%	69%	72%	53%	69%	45%	62%
Part-time	24%	26%	16%	13%	49%	26%	25%	24%	19%	15%	50%	26%
Employment of the father before birth												
Full-time	91%	94%	91%	88%	83%	89%	95%	94%	91%	83%	81%	89%
Part-time	4%	2%	4%	4%	13%	5%	1%	4%	3%	4%	17%	6%

* P<0·05 total active surveillance versus total passive (without active) cohort

sites abbreviations correspond to abbreviations of country names: SCO for Scotland, ENG for England, ESP for Spain, FIN for Finland and NLD for the Netherlands.

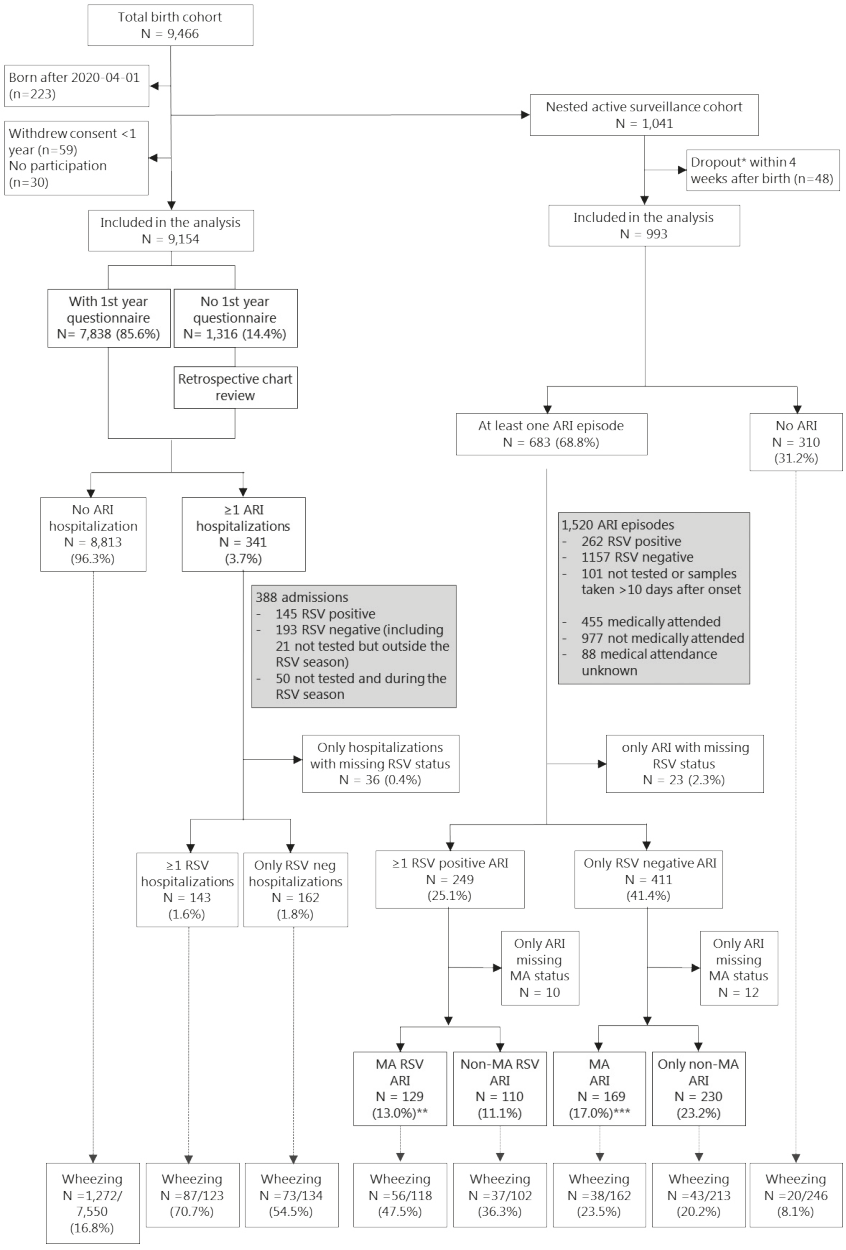

Figure 1. Flow chart of participants in RESCEU birth cohort study for total cohort and active surveillance cohort.

Notes Abbreviations; N= Number of infants

Wheezing: number of children with wheezing of total number of children with known wheezing status

* Dropout: did not continue with active surveillance

** Including 16 RSV admissions (also counted in RSV admissions)

*** Including 7 ARI admissions (also counted in RSV neg admission)

Table 2: Incidence and incidence rates of RSV-associated ARI, MA-ARI and hospitalized ARI in the first year of life

	RSV incidence after imputation[$]	RSV incidence before imputation[$$]	Cohort size / person-time	Number of hospitalizations/ ARI episodes	Number of RSV-positive (observed)	Number of missings[#] (required imputation)
RSV-associated hospitalization in total cohort						
Incidence*	1·8% (1·6-2·1)	1·6% (1·3-1·8)	9,154 infants	341 infants hospitalized	143 infants with RSV-associated hospitalization	50/388 hospitalizations (12.9%)
Incidence rate per 1,000 infant-months**	1·6 (1·3-1·8)	1·3 (1·1-1·6)	109,507 infants-months	388 hospitalizations	145 RSV-associated hospitalizations	
MA RSV-positive ARI in active surveillance cohort						
Incidence*	14·1% (12·3-16·0)	13·0% (11·0-15·2)	993 infants	683 infants with ARI	129 infants with RSV-associated MA-ARI	166/1520 ARI (10.9%)
Incidence rate per 1,000 infant-months**	12·1 (10·2-14·3)	11·2 (9·3- 13·3)	11,728 infant-months	1520 ARI	131 RSV associated MA-ARI	
RSV-positive ARI in active surveillance cohort						
Incidence*	26·2% (24·0-28·6)	25·1% (22·4-27·9)	993 infants	683 infants with ARI	249 infants with RSV-associated ARI	101/1520 ARI (6.7%)
Incidence rate per 1,000 infant-months**	23·7 (21·0-26·7)	22·3 (19·7-25·2)	11,728 infant-months	1520 ARI	262 RSV-associated ARI	

* Incidence as proportion infants experiencing the event at least once during their first year of life. ** Incidence rate as number of events per 1000 infant-months of follow-up.

$ Missing RSV status imputed using multiple imputation based on site, gender, age and meteorological season at time of hospitalization or ARI and missing medical attendance imputed using site, gender, age, meteorological season at time of hospitalization or ARI and RSV status (observed or imputed) $$ assuming all missing outcomes were negative.

Outcomes that required imputations included: 50 hospitalizations with missing RSV status, 166 ARI episodes with missing RSV status and/or missing MA status, 101 ARI episodes with missing RSV status.

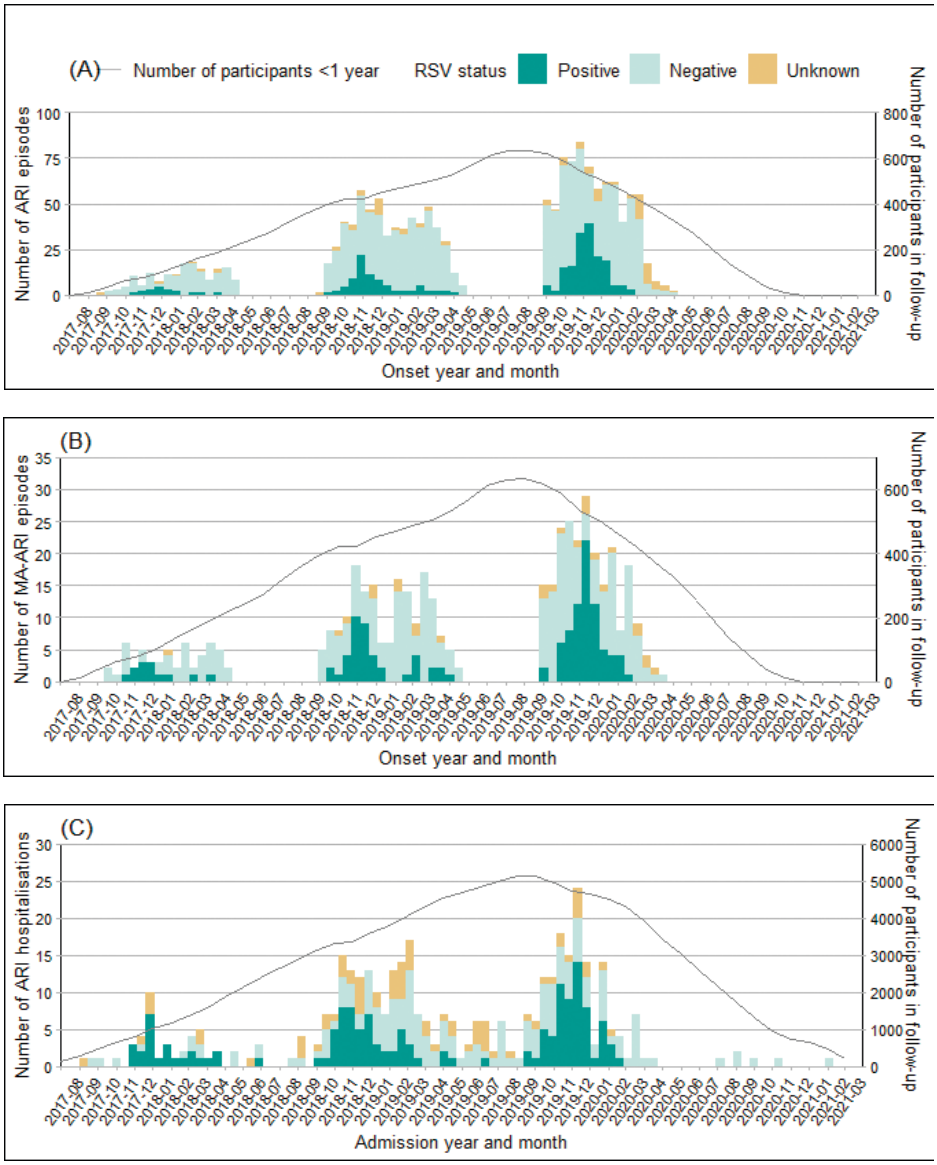

Figure 2. Number of all-cause and RSV-associated ARI by months for ARI (A), MA-ARI (B) and hospitalized ARI (C). Figure (A) and (B) are derived from the active surveillance cohort, figure (C) from the passive surveillance cohort.

Out of 145 RSV-associated hospitalisations, 84 (57·9%) were in children younger than 3 months (appendix). In that age group, incidence of RSV-associated hospitalisations peaked at 1 month to less than 2 months of age (appendix). Median duration of hospitalisation was 3 days (range 1–19 days, IQR 2–5 days). Hospitalisations lasted longer in Spain (median 6 days, IQR 5–6 days) than in the Netherlands (median 3 days, IQR 2–6 days; p<0·003), Finland (median 2 days, IQR 1–4 days), England (median 3 days, IQR 2–4 days), and Scotland (median 2 days, IQR 1–3 days; p<0·001). Duration of hospitalisation and other measures of severity were not found to be associated with the incidence rate of RSV-associated hospitalisations. Length of hospitalisation was longer in infants younger than 3 months when compared with infants aged 6 months to younger than 12 months (p=0·004), but not when compared with infants aged 3 months to younger than 6 months (p=0·27). Eight of 145 RSV-associated hospitalisations (5·5%) led to admission to the paediatric intensive care unit (0·09% of total cohort [n=9154 infants]), and three (2%) required mechanical ventilation (0·03% of total cohort). Six of eight infants admitted to the intensive care unit were aged younger than 3 months (median age 1 month). Any respiratory support was more frequently used in RSV-positive than RSV-negative hospitalisations (77 [53·1%] of 145 *vs* 45 [23·3%] of 193, p<0·001).

Coinfections with other respiratory viruses were tested as part of routine care in 85 (58·6%) and found in 34 (23·4%) of 145 RSV-associated hospitalisations. Rhinovirus was most frequently co-detected. In RSV-negative hospitalisations, rhinovirus, influenza, and parainfluenza were the three most prevalent viruses (appendix).

We registered 1520 ARI episodes in 993 infants in the active surveillance cohort (figure 1, 2). A nasal swab was collected during 1442 (94·9%) episodes. Missed episodes was the main reason for not collecting a swab. 23 samples collected later than 10 days after start of symptoms were excluded. Most samples (87·7%) were collected within 7 days after the start of symptoms. In total, 262 (18·5%) of 1419 episodes were positive for RSV in 249 infants (figure 1). Among the 840 episodes tested by PCR and POCT, RSV was detected only by POCT in five (0·6%).

Table 3. Incidence and incidence rates after imputation for missing RSV test results and missing medical attendance status of RSV-associated hospitalized ARI, MA-ARI and ARI by age group, according to season, recruitment site, cohort, and season of birth.

	RSV-associated hospitalized ARI				RSV-associated MA-ARI	
	< 3 months	**3-<6 months**	**6-<12 months**	**<12 months**	**< 3 months**	**3-<6 months**
RSV incidence proportion (% (95%CI))						
Overall	0·97 (0·82-1·16)	0·49 (0·38-0·63)	0·39 (0·29-0·52)	1·80 (1·58-2·05)	3·39 (2·56-4·49)	4·55 (3·55-5·80)
Site						
Scotland	1·15 (0·83-1·6)	0·47 (0·28-0·79)	0·73 (0·48-1·1)	2·31 (1·83-2·92)	1·48 (0·59-3·64)	5·72 (3·55-9·11)
England[#]	1·03 (0·71-1·51)	0·71 (0·44-1·14)	0·43 (0·23-0·81)	1·97 (1·50-2·57)	2·58 (1·26-5·20)	5·05 (2·97-8·46)
Spain	1·2 (0·77-1·88)	1·00 (0·6-1·65)	0·28 (0·11-0·69)	2·48 (1·81-3·4)	6·00 (3·77-9·43)	6·65 (4·27-10·21)
Finland	0·62 (0·4-0·97)	0·24 (0·12-0·49)	0·19 (0·08-0·44)	1·05 (0·74-1·49)	1·00 (0·33-2·98)	1·01 (0·33-2·99)
Netherlands	0·97 (0·65-1·43)	0·26 (0·12-0·57)	0·25 (0·11-0·56)	1·47 (1·07-2·03)	6·04 (3·73-9·63)	4·28 (2·43-7·43)
RSV incidence rate (/1000 months (95%CI))						
Overall	3·26 (2·63-4·04)	1·67 (1·23-2·27)	0·65 (0·45-0·92)	1·56 (1·33-1·82)	11·69 (8·34-16·38)	15·21 (11·28-20·52)
Site						
Scotland	3·88 (2·60-5·8)	1·55 (0·82-2·92)	1·21 (0·73-2·00)	1·96 (1·48-2·61)	4·95 (1·6-15·35)	19·1 (10·63-34·32)
England	3·46 (2·20-5·45)	2·56 (1·47-4·47)	0·72 (0·34-1·51)	1·87 (1·38-2·55)	8·61 (3·58-20·71)	17·00 (8·89-32·54)
Spain	4·01 (2·33-6·9)	3·34 (1·81-6·14)	0·46 (0·15-1·44)	2·07 (1·41-3·03)	20·11 (11·37-35·55)	22·22 (12·92-38·24)
Finland	2·07 (1·20-3·56)	0·80 (0·33-1·92)	0·31 (0·1-0·9)	0·87 (0·57-1·33)	3·34 (0·84-13·35)	3·35 (0·84-13·41)
Netherlands	3·23 (2·02-5·18)	0·86 (0·33-2·27)	0·40 (0·14-1·15)	1·23 (0·83-1·81)	21·93 (12·46-38·57)	14·27 (7·14-28·54)
Season						
2017-2018	3·9 (2·51-6·08)	2·49 (1·21-5·09)	0***	2·71 (1·85-3·98)	15·01 (7·81-28·86)	11·98 (4·49-31·94)
2018-2019	3·17 (2·30-4·38)	1·41 (0·83-2·41)	0·90 (0·50-1·62)	1·76 (1·38-2·25)	8·36 (4·75-14·71)	9·79 (5·50-17·46)
2019-2020	3·03 (2·1-4·36)	1·79 (1·17-2·76)	0·74 (0·47-1·15)	1·45 (1·14-1·83)	14·90 (8·66-25·64)	21·24 (14·44-31·24)
Cohort						
Cohort A	2·92 (1·48-5·77)	2·45 (1·13-5·29)	0·72 (0·27-1·91)	1·71 (1·08-2·69)		
Cohort P without cohort A	3·30 (2·63-4·14)	1·57 (1·13-2·19)	0·64 (0·44-0·93)	1·54 (1·30-1·82)		
Sex						
Female	3·16 (2·31-4·32)	1·44 (0·9-2·3)	0·55 (0·32-0·93)	1·42 (1·13-1·8)	10·68 (6·45-17·71)	11·37 (6·94-18·63)
Male	3·38 (2·53-4·51)	1·89 (1·24-2·88)	0·74 (0·47-1·17)	1·69 (1·37-2·08)	12·65 (8·05-19·86)	18·82 (12·87-27·52)
Season of birth**						
Spring	0·47 (0·15-1·45)	0·77 (0·31-1·95)	1·02 (0·56-1·83)	0·82 (0·51-1·31)	0***	6·15 (2·45-15·4)
Summer	1·55 (0·86-2·8)	4·24 (2·92-6·15)	0·29 (0·10-0·82)	1·6 (1·18-2·16)	8·17 (3·90-17·14)	36·82 (25·64-52·88)
Fall	8·53 (6·60-11·04)	1·35 (0·7-2·61)	0·17 (0·04-0·65)	2·57 (2·03-3·25)	31·56 (20·95-47·55)	11·37 (5·69-22·73)
Winter	2·03 (1·18-3·48)	0·15 (0·02-1·05)	1·17 (0·7-1·95)	1·13 (0·78-1·62)	7·23 (2·71-19·29)	0***
Birthweight						
<2500 g	5·78 (1·86-17·91)	0***	0***	1·49 (0·48-4·63)	0***	38·45 (11·15-132·56)
≥2500 g	3·18 (2·55-3·96)	1·69 (1·25-2·3)	0·66 (0·47-0·95)	1·55 (1·32-1·82)	12·04 (8·59-16·88)	14·72 (10·77-20·12)

* p<0·05 between groups, cohort P = passive surveillance cohort, cohort A = active surveillance cohort;

** season of birth was defined as follows: spring from March 21st to June 20th, summer from June 21st to September 20th, autumn from September 21st to December 20th, winter from December 21st to March 20th;

*** IR estimated as 0, 95% CI not determined because of 0 cases

			RSV-associated ARI			
6-<12 months	**<12 months**	**< 3 months**	**3-<6 months**	**6-<12 months**	**<12 months**
6·32 (5·13-7·77)	14·07 (12·31-16·03)	5·05 (4·01-6·33)	9·29 (7·84-10·97)	12·61 (10·93-14·51)	26·22 (23·95-28·63)
6·75 (4·30-10·45)	13·74 (10·17-18·31)	3·5 (1·91-6·33)	12·69 (9·17-17·3)	13·6 (9·88-18·43)	29·21 (24·05-34·97)
3·03 (1·48-6·09)	10·4 (7·18-14·84)	3·99 (2·21-7·11)	9·95 (6·89-14·15)	7·61 (4·93-11·55)	20·51 (15·96-25·94)
5·35 (3·22-8·76)	17·71 (13·65-22·65)	7·71 (5·10-11·49)	11·15 (7·98-15·37)	11·8 (8·50-16·16)	29·56 (24·49-35·19)
4·95 (2·95-8·19)	6·9 (4·48-10·49)	1·00 (0·33-2·98)	2·51 (1·23-5·07)	7·07 (4·62-10·68)	10·50 (7·45-14·61)
11·66 (8·32-16·10)	21·98 (17·38-27·39)	9·25 (6·30-13·38)	10·16 (7·08-14·38)	23·32 (18·6-28·81)	42·19 (36·35-48·26)
10·77 (8·36-13·88)	12·11 (10·24-14·34)	17·55 (13·34-23·1)	31·69 (25·76-38·98)	22·81 (19·16-27·17)	23·7 (21·02-26·73)
11·47 (6·62-19·87)	11·75 (8·06-17·12)	11·70 (5·58-24·56)	44·82 (30·18-66·56)	24·77 (16·78-36·56)	26·52 (20·54-34·25)
5·04 (2·09-12·1)	8·98 (5·69-14·18)	13·31 (6·44-27·51)	34·07 (21·68-53·55)	12·99 (7·63-22·1)	18·39 (13·4-25·23)
8·93 (4·8-16·61)	15·09 (10·82-21·06)	27·46 (16·81-44·88)	37·28 (24·56-56·59)	20·58 (13·77-30·75)	26·49 (20·63-34·03)
8·24 (4·37-15·52)	5·79 (3·4-9·85)	3·34 (0·84-13·35)	8·38 (3·49-20·14)	11·78 (6·98-19·89)	8·81 (5·74-13·51)
20·28 (13·43-30·64)	19·2 (14·21-25·93)	32·63 (20·57-51·77)	33·9 (21·62-53·15)	44·48 (33·62-58·85)	38·89 (31·49-48·02)
0***	12·05 (7·00-20·75)	20·75 (11·75-36·67)	18·08 (8·03-40·72)	0***	17·15 (10·79-27·26)
10·37 (6·64-16·19)	9·60 (7·12-12·95)	12·10 (7·56-19·38)	20·32 (13·60-30·37)	21·3 (15·62-29·05)	18·19 (14·67-22·55)
12·65 (9·26-17·29)	15·06 (12·04-18·83)	24·32 (15·89-37·22)	46·16 (35·79-59·54)	27·2 (21·99-33·66)	31·25 (26·81-36·42)
11·49 (8·07-16·37)	11·26 (8·77-14·46)	17·39 (11·66-25·92)	28·39 (20·73-38·89)	23·99 (18·8-30·61)	23·43 (19·71-27·84)
10·09 (7·04-14·48)	12·92 (10·31-16·19)	17·73 (12·08-26·03)	34·16 (25·81-45·21)	21·72 (16·98-27·78)	23·82 (20·16-28·14)
18·52 (12·77-26·86)	10·72 (7·60-15·12)	0***	16·71 (9·70-28·77)	42·87 (33·49-54·87)	25·43 (20·31-31·83)
2·03 (0·65-6·3)	12·32 (9·01-16·83)	14·99 (8·66-25·95)	78·13 (61·17-99·79)	4·92 (2·39-10·15)	25·81 (20·85-31·95)
1·48 (0·37-5·91)	11·55 (8·19-16·27)	46·95 (33·56-65·67)	17·83 (9·98-31·88)	4·22 (1·90-9·4)	18·41 (13·99-24·23)
25·22 (17·4-36·55)	14·41 (10·17-20·41)	7·23 (2·71-19·29)	0***	46·33 (35·24-60·9)	24·97 (19·17-32·51)
6·94 (0·98-49·29)	13·42 (4·87-36·98)	0***	72·07 (30·01-173·09)	7·44 (1·05-52·97)	22·04 (9·96-48·75)
10·94 (8·47-14·13)	12·16 (10·25-14·43)	18·1 (13·75-23·82)	30·54 (24·63-37·87)	23·17 (19·43-27·62)	23·73 (21·01-26·81)

RSV-A was detected in 142 (54·2%) of RSV-associated ARI and RSV-B in 111 (42·4%). One sample was positive for both RSV-A and RSV-B. RSV subtype was unknown for ten ARI episodes: five were only tested by POCT, four were only tested in hospital as part of routine care, and for one the RSV subtype could not be determined. Information about medical attendance was available for 1432 (94·2%) episodes. For 1353 (89·0%) ARI episodes both RSV and medical attendance status were available. Medical attendance was reported in 131 (52·2%) of 251 RSV-positive ARI, which was more frequent than in RSV-negative ARI (298 [27·0%] of 1102, p<0·001). ARI and medically attended RSV-associated ARI episodes were highest in the Netherlands (38·9 per 1000 infant-months [31·5–48·0] and 19·2 per 1000 infant-months [14·2–25·9], respectively) and lowest in Finland (8·8 per 1000 infant-months [5·7–13·5] and 5·8 per 1000 infant-months [3·4–9·9] respectively, Bonferroni adjusted p<0·05; table 3).

Information on wheezing was available for 7838 children whose parents completed the 1-year questionnaire (85·6% of the 9154 participants), which included 7807 participants of the total cohort with complete information on hospitalisations for ARI and 841 participants of the active surveillance cohort with complete information on ARI episodes (figure 1). Wheezing was reported in 87 (70·7%) of 123 infants admitted with RSV. Wheezing was less frequent in infants hospitalised for RSV-negative ARI only (73 [54·5%] of 134, p=0·008) and in infants never admitted for an ARI (1272 [16·8%] of 7550, p<0·001, figure 1). In the active surveillance cohort, wheezing was reported for 56 (47·5%) of 118 infants with medically attended RSV-associated ARI and 37 (36·3%) of 102 infants with non-medically attended RSV-associated ARI (p=0·09). This occurrence was more frequent than in children who had no ARI (20 [8·1%] of 246, p<0·001 and p<0·001), had medically attended RSV-negative ARI (38 [23·5%] of 162, p<0·001 and p=0·03) or had non-medically attended RSV-negative ARI (43 [20·2%] of 213, p<0·001 and p=0·002). When adjusted for family history of atopy and smoking household members at birth, the difference in wheezing between RSV-positive and RSV-negative or no ARI remained significant (p=0·003 and p<0·001 for hospitalisations, p<0·001 and p<0·001 for medically attended ARI, and p=0·002 and p<0·001 for non-medically attended ARI).

DISCUSSION

To our knowledge, this is the first international birth cohort study powered to accurately estimate the health-care burden of RSV in healthy term-born infants. Our results showed an incidence of RSV-associated hospitalisation of 1·8% in the first year of life. Almost half of all ARI hospitalisations in the first year of life were RSV-associated. The burden of RSV-associated hospitalisation was highest in infants younger than 3 months with an incidence rate of 3·3 per 1000 infant-months. Children born in autumn had a significantly higher risk of hospitalisation than children born in other seasons. One quarter of infants experienced an RSV-associated ARI, of which half were medically attended. Wheezing during the first year of life was associated with RSV hospitalisation, medically attended RSV-associated ARI, and overall RSV-associated ARI.

Our findings are consistent with previous literature. Although not a birth cohort study, a study conducted in the USA reported an incidence of RSV-associated hospitalisations of 1·7% in infants younger than 6 months (1·5% in our study), and 0·5% in infants aged 6 to younger than 12 months (0·4% in our study).2 The higher admission rate in infants younger than 6 months reported by Hall and colleagues2 might be related to the 35% of higher-risk infants included. In our study, incidence of RSV-associated hospitalisations per country varied between 1·1% and 2·5%, which was in line with previous findings from these countries.9,11,18,22 In other birth cohort studies, RSV-associated hospitalization incidence in the first year of life varied between 0·6% and 5%. Some studies also included high-risks infants (appendix).10,12–17 The two largest birth cohort studies in healthy term-born infants showed an incidence of RSV-associated hospitalisations of 1·9% in an Indian birth cohort of 310 infants and 1% in 298 infants of a Dutch birth cohort.9,14

Wheezing in the first year of life was associated with RSV infection irrespective of severity. The association between severe RSV infections and wheezing has been described earlier.23 Whether this is also associated with development of childhood asthma remains unclear, as well as whether RSV immunisation will prevent wheezing during later childhood.24 Intervention studies are required to define the causal association between RSV infection during infancy and wheezing in healthy term-born infants.

The major strength of our study is the prospective design with the power to accurately estimate RSV incidence in European countries over several seasons. We used active surveillance to capture mild RSV disease to provide a precise estimate of total RSV incidence and disease burden. Follow-up rates were high with collection of swabs in 95% of reported ARI episodes and more than 85% completion of the 1-year questionnaire in the total cohort. In addition to parental report, we screened the study participants' hospital charts to ensure no ARI hospitalisation was missed. This study also has limitations. First, in 50 of 388 ARI hospitalisations during the RSV season, no RSV test was performed. When using a cohort study design with RSV testing results as primary outcome, missing test results will systematically lead to an underestimation of true incidence if assumed negative. To avoid this systematic bias, primary outcomes were reported after using multiple imputation for missing RSV test results and medical attendance status. As the proportion of missing information was small, using multiple imputation resulted in a small increase in incidence compared with estimating incidence assuming all cases with missing RSV status were RSV-negative. Two of the five sites did not use POCT, which could have led to underestimating incidence in those countries; however, that effect was probably small. Of 840 episodes tested by PCR and POCT, five (0·6%) were detected by POCT only. Assuming a similar rate, two additional RSV cases would have been detected by POCT among the 415 episodes tested by PCR only at the sites not using POCT. Second, data on coinfection with other respiratory viruses were scarce. Third, the participants in the study might not be representative of the country population and not all countries in Europe were represented. The education level of participants, especially in the active surveillance cohort, was high with 70% of mothers reporting university education and is therefore not necessarily representative of the whole population. Lower socioeconomic status and younger age of the mother have been reported as risk factors for RSV-associated hospitalisation in infancy.25 Other risk factors like parental smoking were less frequently reported by active surveillance cohort participants than the rest of the study population. This could have resulted in an underestimation of RSV incidence in the study population compared with the country population and in the active cohort compared with the entire cohort. Although children with evidence of significant comorbidities at birth were excluded, we cannot rule out that a minority of participants had comorbidities diagnosed later in life. Fourth, it is possible that we missed ARI episodes despite weekly contacts with parents during the period of active surveillance (October to May, or longer if RSV was still circulating). We cannot rule out that some participants could have stopped

reporting ARI of their children, which could result in underestimating incidence rate and would be more pronounced in the older infants. However, participation to the 1-year questionnaire was 89% in the active surveillance cohort, suggesting a high retention rate. ARI episodes occurring outside of the active surveillance period would not have been captured, which probably contributed to the finding of 31% of active cohort participants with no ARI in the first year of life. However, it is unlikely that those uncaptured ARI episodes were associated with RSV infection. Fifth, the COVID-19 pandemic impacted RSV incidence in 2020. The 2019–20 RSV season was virtually finished in the participating countries when the COVID-19 pandemic started, except for Finland, where the usual continuation of the RSV outbreak into late spring was abruptly terminated because of the COVID-19 pandemic.26,27 The COVID-19 pandemic might have contributed to the lower incidence of RSV-associated hospitalisations, medically attended ARIs, and RSV-associated ARIs in the study in Finland. Participants born after April 1, 2020, were excluded as RSV did not circulate during their first year of life. Follow-up time after Nov 1, 2020, represented less than 3% of total follow-up time of the cohort and concerned only participants aged 6 months or older. Sixth, health-care burden does not reflect the total burden of RSV. Health-care burden is key information to estimate economic and societal burden, and the incidence of medically attended and hospitalised RSV infections is expected to be a major part of the health-care burden in Europe where RSV-related deaths are rare. Overall, study limitations have possibly resulted in a modest underestimation of actual RSV burden.

Conclusions

In conclusion, the health-care burden of RSV in healthy term-born infants in Europe is considerable with an incidence of RSV-associated hospitalisation of 1·8% in the first year of life, which means that one in 56 healthy term-born infants is hospitalised with RSV annually. Because the highest burden is seen in infants in their first months of life, maternal vaccination and passive immunisation could have a profound impact on the RSV burden.

NOTES

Study group members

The RESCEU investigators are as follows: Joanne Wildenbeest; Marie-Noëlle Billard; Roy Zuurbier; Koos Korsten; Marlies van Houten; Annefleur Langedijk; Peter ; Louis Bont (University Medical Center Utrecht); Simon Drysdale; Joseph McGinley; Gu-Lung Lin; Matthew Snape; Andrew Pollard, Andrew Ives, Helen Wolfenden, Sanjay Salgia, Rohoth Shetty (University of Oxford); Ana Dacosta-Urbieta; Irene Rivero-Calle; Alberto Gómez-Carballa; Sara Pischedda; Carmen Rodriguez-Tenreiro; Federico Martinón-Torres (Servicio Galego de Saude); Terho Heikkinen (University of Turku and Turku University Hospital); Steve Cunningham; Harish Nair; Harry Campbell (University of Edinburgh); Thomas O'Neill; Margaret Miller; Julie Baggott; Catherine Beveridge; Rachael McKernan (Children's Clinical Research Facility, NHS Lothian, Edinburgh); Bishoy Rizkalla (GlaxoSmithKline) Philippe Beutels (University of Antwerp); Peter Openshaw (Imperial College London); Adam Meijer (National Institute for Public Health and the Environment); Thea Kølsen Fischer (Statens Serum Institut); Maarten van den Berge (Academisch Ziekenhuis Groningen); Carlo Giaquinto (Fondazione PENTA for the Treatment and Care of Children with HIV-ONLUS); Michael Abram (AstraZeneca); Kena Swanson (Pfizer); Jeroen Aerssens (Janssen); Charlotte Vernhes, Scott Gallichan (Sanofi Pasteur); Veena Kumar (Novavax); Eva Molero (Team-It Research).

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Conflict of interests

LJB has regular interaction with pharmaceutical and other industrial partners. He has not received personal fees or other personal benefits. UMCU has received major funding (>€100,000 per industrial partner) for investigator initiated studies from AbbVie, MedImmune, Janssen, the Bill and Melinda Gates Foundation, Nutricia (Danone) and MeMed Diagnostics. UMCU has received major cash or in kind funding as part of the public private partnership IMI-funded RESCEU project from GSK, Novavax, Janssen, AstraZeneca, Pfizer and Sanofi. UMCU has received major funding by Julius Clinical for participating in the INFORM study sponsored by MedImmune. UMCU has received minor funding for participation in trials by Regeneron and

Janssen from 2015-2017 (total annual estimate less than €20,000). UMCU received minor funding for consultation and invited lectures by AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, Novavax, Pfizer, Janssen (total annual estimate less than €20,000). Dr. Bont is the founding chairman of the ReSViNET Foundation. SC has provided consultancy and/or investigator roles in relation to product development for Ablynx, Janssen, MedImmune, AstraZeneca, Pfizer, GSK, Vertex, AbbVie, Valneva, Fibrogen, Boehringer Ingelheim, with fees paid to the University of Edinburgh. FM-T has received honoraria from GSK group of companies, Pfizer Inc, Sanofi Pasteur, MSD, Seqirus, Biofabri and Janssen for taking part in advisory boards and expert meetings and for acting as a speaker in congresses outside the scope of the submitted work. FM-T has also acted as principal investigator in randomized controlled trials of the above-mentioned companies as well as Ablynx, Gilead, Regeneron, Roche, Abbott, Novavax, and MedImmune, with honoraria paid to his institution.

MDS acts as an investigator on behalf of the University of Oxford on research studies funded by vaccine manufacturers including GlaxoSmithKline, Janssen, MCM vaccines, Novavax, AtraZeneca and Pfizer. He receives no direct personal payment for this work. MDS was an NIHR senior Investigator and received salary support from the NIHR Oxford Biomedical Research Centre during the course of this project. MDS is currently an employee of Moderna. SBD had received honoraria from MSD and Sanofi Pasteur for taking part in advisory boards and has provided consultancy and/or investigator roles in relation to product development for Janssen, AstraZeneca, Pfizer, Valneva, MSD and Sanofi Pasteur with fees paid to St George's University of London. TH has received honoraria for lectures and/or participation in advisory boards or data monitoring committees from Janssen, Sanofi Pasteur, Enanta and MSD. BR is a full time employee of the GSK group of companies and holds shares and restricted shares in the GSK group of companies as part of their employee remuneration. AJP is currently Chair of DHSC's JCVI and was previously a member of WHO's SAGE and chair of the European Medicine's Agency Scientific Advisory Group on Vaccines. Oxford University has partnered with AstraZeneca on development of COVID19 vaccines. Other authors declare no conflict of interests.

Disclaimer

This manuscript reflects only the views of the authors. The European Union and the Innovative Medicines Initiative (IMI) are not responsible for any use that may be made of the information it contains.

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Author contributions

JGW, AP, TH, SC, FMT, MS and LJB designed the study. JGW, RZ, MvH, TH, SC, MS, SC, FMT, KK, SD, HR, ADU and TON collected data. JGW, MB, PvdV, and LJB analysed and interpreted data. JGW wrote the first draft. AP, TH, SC, FMT, MS, RZ, MvH, KK, SD, HR, ADU, BR and TON reviewed and commented on the manuscript. JGW and MB accessed and verified the data. JGW and LJB were responsible for the decision to submit the manuscript.

Data sharing statement

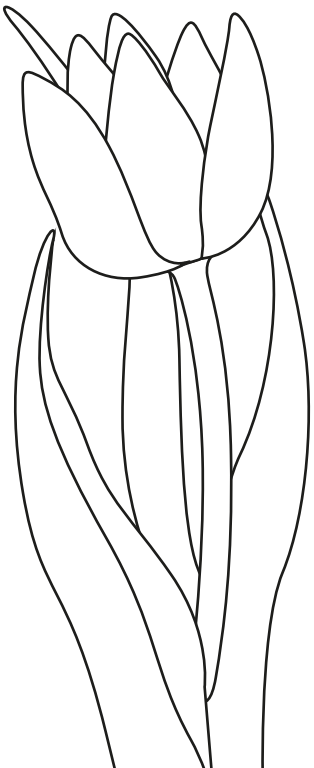
The anonymized data of the RESCEU birth cohort study will be made available for research purposes after the end of the long-term follow-up. The data will be store on the Elixir data platform. Requests to access the data should be sent via Elixir to the RESCEU consortium.

REFERENCES

1. Li Y, Wang X, Blau DM, *et al.* Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *The Lancet* 2022; **399**: 2047–64.
2. Hall CB, Weinberg GA, Iwane MK, *et al.* The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 2009; **360**: 588–98.
3. Madhi SA, Polack FP, Piedra PA, *et al.* Respiratory Syncytial Virus Vaccination during Pregnancy and Effects in Infants. *New England Journal of Medicine* 2020; **383**: 426–39.
4. Hammitt LL, Dagan R, Yuan Y, *et al.* Nirsevimab for Prevention of RSV in Healthy Late-Preterm and Term Infants. *N Engl J Med* 2022; **386**: 837–46.
5. RSV Vaccine and mAb Snapshot. https://www.path.org/resources/rsv-vaccine-and-mab-snapshot/ (accessed March 22, 2022).
6. EMA. New medicine to protect babies and infants from respiratory syncytial virus (RSV) infection. European Medicines Agency. 2022; published online Sept 16. https://www.ema.europa.eu/en/news/new-medicine-protect-babies-infants-respiratory-syncytial-virus-rsv-infection (accessed Sept 26, 2022).
7. Haerskjold A, Kristensen K, Kamper-Jørgensen M, Nybo Andersen A-M, Ravn H, Graff Stensballe L. Risk Factors for Hospitalization for Respiratory Syncytial Virus Infection: A Population-based Cohort Study of Danish Children. *Pediatr Infect Dis J* 2016; **35**: 61–5.
8. Hardelid P, Verfuerden M, McMenamin J, Smyth RL, Gilbert R. The contribution of child, family and health service factors to respiratory syncytial virus (RSV) hospital admissions in the first 3 years of life: birth cohort study in Scotland, 2009 to 2015. *Euro Surveill* 2019; **24**. DOI:10.2807/1560-7917.ES.2019.24.1.1800046.
9. Houben ML, Bont L, Wilbrink B, *et al.* Clinical prediction rule for RSV bronchiolitis in healthy newborns: prognostic birth cohort study. *Pediatrics* 2011; **127**: 35–41.
10. Nokes DJ, Okiro EA, Ngama M, *et al.* Respiratory syncytial virus infection and disease in infants and young children observed from birth in Kilifi District, Kenya. *Clin Infect Dis* 2008; **46**: 50–7.
11. Toivonen L, Karppinen S, Schuez-Havupalo L, *et al.* Respiratory syncytial virus infections in children 0-24 months of age in the community. *J Infect* 2020; **80**: 69–75.
12. Kubale J, Kuan G, Gresh L, *et al.* Assessing the Incidence of Symptomatic Respiratory Syncytial Virus Illness Within a Prospective Birth Cohort in Managua, Nicaragua. *Clin Infect Dis* 2020; **70**: 2029–35.
13. Zar HJ, Nduru P, Stadler JAM, *et al.* Early-life respiratory syncytial virus lower respiratory tract infection in a South African birth cohort: epidemiology and effect on lung health. *The Lancet Global Health* 2020; **8**: e1316–25.
14. Kumar P, Medigeshi GR, Mishra VS, *et al.* Etiology of Acute Respiratory Infections in Infants: A Prospective Birth Cohort Study. *Pediatr Infect Dis J* 2017; **36**: 25–30.
15. Regamey N, Kaiser L, Roiha HL, *et al.* Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J* 2008; **27**: 100–5.
16. Kusel MMH, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006; **25**: 680–6.
17. Takashima MD, Grimwood K, Sly PD, *et al.* Epidemiology of respiratory syncytial virus in a community birth cohort of infants in the first 2 years of life. *Eur J Pediatr* 2021; **180**: 2125–35.

18. Thomas E, Mattila J-M, Lehtinen P, Vuorinen T, Waris M, Heikkinen T. Burden of Respiratory Syncytial Virus Infection During the First Year of Life. *J Infect Dis* 2021; **223**: 811–7.
19. Wildenbeest JG, Zuurbier RP, Korsten K, *et al.* Respiratory Syncytial Virus Consortium in Europe (RESCEU) Birth Cohort Study: Defining the Burden of Infant Respiratory Syncytial Virus Disease in Europe. *The Journal of Infectious Diseases* 2020; **222**: S606–12.
20. Korsten K, Adriaenssens N, Coenen S, *et al.* Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study. *Eur Respir J* 2021; **57**: 2002688.
21. Zuurbier RP, Korsten K, Verheij TJM, *et al.* Performance Assessment of a Rapid Molecular Respiratory Syncytial Virus Point-of-Care Test: A Prospective Community Study in Older Adults. *J Infect Dis* 2022; **226**: S63–70.
22. Zomer-Kooijker K, Uiterwaal CSPM, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. *Eur Respir J* 2014; **44**: 666–74.
23. Blanken MO, Rovers MM, Molenaar JM, *et al.* Respiratory Syncytial Virus and Recurrent Wheeze in Healthy Preterm Infants. *N Engl J Med* 2013; **368**: 1791–9.
24. Brunwasser SM, Snyder BM, Driscoll AJ, *et al.* Assessing the strength of evidence for a causal effect of respiratory syncytial virus lower respiratory tract infections on subsequent wheezing illness: a systematic review and meta-analysis. *The Lancet Respiratory Medicine* 2020; **8**: 795–806.
25. Fitzpatrick T, McNally JD, Stukel TA, *et al.* Family and Child Risk Factors for Early-Life RSV Illness. *Pediatrics* 2021; **147**: e2020029090.
26. Haapanen M, Renko M, Artama M, Kuitunen I. The impact of the lockdown and the re-opening of schools and day cares on the epidemiology of SARS-CoV-2 and other respiratory infections in children - A nationwide register study in Finland. *EClinicalMedicine* 2021; **34**: 100807.
27. van Summeren J, Meijer A, Aspelund G, *et al.* Low levels of respiratory syncytial virus activity in Europe during the 2020/21 season: what can we expect in the summer and autumn/winter? *Eurosurveillance* 2021; **26**: 2100639.

If I don't get some shelter
Ooh yeah I'm gonna fade away
(The Rolling Stones – Gimme Shelter)



Chapter 7

The burden of respiratory syncytial virus and influenza associated acute respiratory tract infection in infants – a prospective birth cohort study

Roy P. Zuurbier, Marie. N. Billard, Louis J. Bont, Koos Korsten, Pieter-Kees C.M. de Groot, Anna E. de Jong, Sjoerd Euser, Bjorn L. Herpers, Joanne G. Wildenbeest, Marlies A. van Houten, for the RESCEU Investigators

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ABSTRACT

Background. Respiratory syncytial virus (RSV) and influenza are leading causes of respiratory tract infections in infants. In contrast to influenza, for which antiviral therapy and vaccines are available, no treatment or vaccine is currently available for RSV. RSV prevention and therapeutics for infants is expected to be available soon. The need for prioritization of preventive and therapeutic strategies against these respiratory viruses in infants is necessary because of limited resources. The aim of the study is to compare the burden of influenza versus RSV infections in infants (<1 year).

Methods. Data of two prospective studies, a birth cohort and a hospital cohort, consisting of (previously) healthy infants followed during three RSV season (2017-2020) were combined. In the birth cohort study a respiratory swab was collected each time participants experienced an ARTI (acute respiratory tract infection) and parents completed a symptom diary. In the hospital cohort, a swab was collected from all healthy infants who were admitted to the pediatric ward of a secondary hospital because of an ARTI and data was retrieved from patient records. All samples were tested on a broad panel of viruses and subtypes with a qualitative multiplex real-time PCR.

Results. The birth cohort consisted of 187 infants, in which 457 swabs were collected during their first year of life. An overall incidence of 3.2% (6/187) for influenza-ARTI and 35.8% (67/187) for RSV-ARTI was found. Influenza-ARTI resulted in less medical consultation compared with RSV-ARTI (16.7% [1/6] vs 53.8% [36/67]; aOR: 4.1, 95% CI 0.62 – 27.20). For the hospital cohort, 324 swabs were taken in 304 infants, RSV was the most common detected virus (49.3%; 150/304), influenza was the third most common virus detected (7.6%; 23/304).

Conclusion. RSV was responsible for the highest number of ARTIs compared to influenza, especially during the first months of life. The incidence of influenza-ARTI was low compared to RSV-ARTI. These findings suggest that RSV preventive and therapeutic strategies could have the highest impact and therefore should be prioritized.

Clinical Trials Registration: NCT03627572

Keywords

Respiratory syncytial virus, influenza, infants, community, prevention

BACKGROUND

Acute respiratory tract infections (ARTIs) are amongst the leading causes of morbidity and mortality in children aged younger than 5 years worldwide [1–3]. ARTIs include conditions as pneumonia, bronchiolitis, bronchitis, influenza and whooping cough; clinical diagnoses with a predominantly viral etiology [4]. Both influenza and respiratory syncytial virus (RSV) are recognized as major pathogens in these infectious diseases [5–7]. In particular, infants are significant contributors to the worldwide high numbers of childhood deaths and hospitalizations due to acute lower respiratory tract infections (ALRIs) [5,6]. Overall incidence of influenza-ARTI in infants from developed countries is estimated at 5.2% [8], for RSV-ARTI this is estimated at 10% [3,9,10], however there is still lack of community data. A safe and effective intervention to protect infants during the first months of life against influenza is maternal vaccination [11]. In 2012, the World Health Organization (WHO) recommended influenza vaccination for pregnant women to protect mothers as well as their infants [12]. Despite this recommendation, the global maternal vaccination rate for influenza is still low [11]. Meanwhile, maternal vaccination against RSV is in phase-3 of clinical development [13,14]. The possibility of an effective RSV vaccine in the (near) future is of great importance for RSV infection prevention in infants, since there are no treatment options for RSV except supportive care. Because of expected limited resources, prioritization of preventive and therapeutic strategies against influenza and RSV is necessary [15].

Therefore, better understanding of the burden of influenza and RSV in infants is needed for immunization recommendations and prioritize research and development funding. Data on burden of disease in healthy term born infants is scarce, since most studies are performed in high-risk groups. For this purpose, our aim in this study is to compare the burden of RSV versus influenza in community and hospitalized healthy term infants.

METHODS

Study population

The study population consisted of healthy term born infants (<1 year old) with an acute respiratory tract infection (ARTI) who were hospitalized or participating in the REspiratory Syncytial virus Consortium in EUrope (RESCEU) [16,17] birth cohort

study during three consecutive RSV seasons between 1 October 2017 and 30 April 2020. The study was a combination of two prospective studies, hereafter called the hospital cohort and birth cohort, respectively. The current study was performed in the Netherlands, where maternal influenza vaccination is not given routinely.

The birth cohort study consists of healthy infants prospectively followed up from birth as part of the RESCEU study, an EU-funded consortium aiming to define RSV burden of disease in Europe. In their first year of life, during the RSV season(s), nasal swab was taken each time they experienced any symptoms of an ARTI. Infants were swabbed by a trained member of the study team at home and could be tested during more than one separate episode, hereafter called study team visit. Informed consent was obtained from the parents of all study participants. Data on age, sex, duration of symptoms of ARTI, and level of medical care needed were obtained by completing questionnaires and case report forms (CRF), including ReSViNET score to determine disease severity [18]. In addition, parents completed a 14-day symptom diary. Three levels of medical care were defined: infants with ARTI who were hospitalized, infants with medically attended (MA) ARTI, defined as infants who were seen at the emergency department (ED) or by a general practitioner (GP), but not admitted to the hospital, and infants with non-MA ARTIs who did not see any doctor during the entire ARTI episode.

The hospital cohort was added to boost robustness on the severity data. Within this cohort, in all previously healthy infants admitted to the pediatric ward with any respiratory symptoms a swab was taken. Patient and clinical characteristics were retrospectively obtained through the electronic health record system, including age, sex, respiratory symptoms, length of stay, ancillary testing, and treatment.

Study procedures

For the birth cohort, a nasal flocked swab (FLOQSwab™, Copan diagnostics) was collected by a trained member of the study team and directly stored in viral transport media: MicroTest™ M4RT® (Remel, 3 ml). A maximum of 200 µl of the viral transport medium was used for viral testing. Samples were transported at room temperature. The remaining sample was stored in aliquots at −80°C.

For the hospital cohort a naso- or oropharyngeal swab was taken by the physician and stored in VTM (eSwab™, Copan diagnostics) and directly transported and tested in the laboratory.

Viral analysis

All samples were tested on viruses with qualitative multiplex real-time PCR (RespiFinder SMARTfast 22 [Maastricht, Netherlands]). DNA and RNA were isolated from 200 µL of both swabs by MagCore isolation and eluted in 60 µL of buffer (RBC Biosciences, Taiwan). Five microliters was used for the detection of respiratory viruses by a qualitative multiplex real-time PCR–based multiplex ligation-dependent probe amplification (MLPA) assay (RespiFinder 2Smart kit; Pathofinder, the Netherlands). All analyses were performed on a Roche Lightcycler 480II. Samples were tested on a panel of eight viruses: respiratory syncytial virus (RSV) A and B, human rhinovirus/enterovirus, influenza A, B and A(H1N1)pdm09, human coronavirus 229E, NL63/HKU1 and OC43, human metapneumovirus (hMPV), parainfluenza virus (PIV) 1 to 4, adenovirus and bocavirus.

Statistical analysis

Statistical analyses were conducted using R version 4.0.2 within RStudio version 1.2.5. A p-value <0.05 was considered statistically significant. Incidence of respiratory viruses in the birth and hospital cohort was calculated as the number of detections divided by the study population. Confidence intervals were calculated using the Exact Clopper-Pearson method. Comparisons between RSV-ARTI and influenza-ARTI, regarding patient and clinical characteristics, were analyzed using generalized estimating equations (GEE) with logit link, including age for multivariable models. In these models, individuals were clustered to adjust for repeated measurements.

RESULTS

Study populations

In the birth cohort 187 participants were included from birth and followed during their first year of life. One (0.5%) participant was lost to follow-up during the active surveillance period. For the hospital cohort, 324 samples were taken in 304 infants who were admitted to the pediatric ward with ARTI symptoms. Baseline characteristics of both cohorts are shown in Table 1. 63.6% (119/187) of the birth cohort participants had at least one sibling (aged younger than 5 years), 76% (142/187) went to daycare

(for at least 8 hours a week) in their first year of life. None of the participants and 2.7% (5/187) of the mothers were vaccinated against influenza.

Table 1. Baseline characteristics for participants of both cohorts. Birth cohort data was obtained by baseline questionnaires and case report forms, and questionnaire at 1st year of life. Hospital cohort data was obtained by patient record data.

	Birth cohort (n = 187)	**Hospital cohort (n = 304)**
Sex, male (%)	85 (45.5)	171 (56.2)
Delivery, C-section (%)	44 (23.5)	49 (23.2)
Birth weight < 2500g (%) (n=183)	3 (1.6)	7 (2.3)
Daycare (%) [in first year of life] (n=150) Weeks (n=123)	136 (76.0) 28 [16 – 35]	NA
Siblings (<5 years of age (%))	119 (63.6)	NA
Breastfeeding (%) (n=154) Weeks of exclusively breastfeeding (n=98)	123 (82.0) 20 [12 – 30]	NA

Acute respiratory tract infections (birth cohort)

In total, 458 ARTIs were reported in 166/187 participants (88.7%, range 1-8 episodes) of the birth cohort. Study team visits resulted in 457 nasal swab samples 99.6% (457/458) of ARTIs; one sample was missed due to hospitalization elsewhere. Median time between onset of symptoms and study visit was 3 days (range 0-19) and 88.9% (51/403) of tested infants were visited within 5 days after onset of symptoms. Diaries by parents were completed for 96.2% (441/458) of the ARTIs, resulting in 24.2% MA-ARTIs (107/441) and thereby 38.0% (71/187) of the infants with at least one MA-ARTI. Eight episodes required hospitalization in seven infants (3.7%; 7/187). Antibiotics were prescribed during nine ARTIs (2.0%; 9/441), none of these infants were hospitalized.

Incidence of respiratory viruses

In total, 92.1% (421/457) of the samples were positive for any virus in the birth cohort. RSV was found in 67/187 participants in their first year of life (35.8%, 95% CI 29.0-43.2%), of which 37.3% (25/67) were RSV B. Three infants were tested twice positive for RSV, of which two within a month and one 4 months later. Influenza was detected in 6/187 participants (3.2%, 95% CI 1.2-6.9%), of which one influenza A, three influenza H1N1, and two influenza B. In one of the cases mother was vaccinated against influenza during pregnancy, however influenza was detected at the age of 11 months. The incidence of RSV and influenza were 16.6% (31/187) and 0.5% (1/187), respectively, during the first 6 months of life.

For the hospital cohort, at least one virus was found in 83.3% (270/324) of the samples. RSV was the most common detected virus in 49.3% (150/304) of the infants, followed by rhinovirus (24.7%; 75/304) and influenza in 7.6% (23/304) (Suppl Table 1). One patient was positive for influenza and RSV. 59.3% (89/150) of the RSV-ARTIs occurred within the first three months of life, for influenza this was 52.2% (12/23; Suppl Figure 1).

Symptoms and severity

Severity of disease in the birth cohort was compared between 441 ARTIs with complete diary data (Table 2 and 3). Median age for influenza infection was 312 days, for RSV it was 198 days. 24.2% (107/441) of the ARTIs required medical consultation. Longitudinal multivariable modelling (GEE) showed that RSV-ARTI (aOR: 4.3, 95% 2.6 – 7.0) was significantly associated with medical consultation compared to non-RSV-ARTIs. In one (17%; 1/6) influenza-ARTI medical consultation was required, no significant association for influenza-ARTI (aOR: 0.80, 95% 0.1 – 5.2) was found compared to non-influenza ARTIs. RSV-ARTI required more medical attendance (53.8% [36/67] vs 16.7% [1/6]; aOR: 4.1, 95% CI 0.62 – 27.20), however not significantly. In none of the infants with an RSV-ARTI antibiotics were prescribed, for influenza this was the case for only one participant. Median symptom duration for RSV-ARTI was 12 days and was similar to influenza-ARTI (11 days). ReSViNET score for influenza-ARTI (score 6) was significantly higher compared to other ARTI (3), but similar with RSV-ARTI (5). Eight ARTI episodes required hospitalization, of which four tested positive for RSV (50%; 4/8). Sufficient diary information was available for 389/462 of ARTIs on symptoms in the birth cohort. There were no significant differences in parental reported symptoms for RSV and influenza compared to other ARTI (Suppl Table 2). Peak of symptoms was for all types of ARTIs at 4 days after onset.

Table 2. Characteristics of ARTIs in birth cohort

	Total (n=458)	Influenza-ARTI (n=6)	RSV-ARTI (n=67)	OR [95% CI]
Median age, days [IQR]	192 [106 – 280]	312 [269 – 331]	198 [111 – 279]	0.99 [0.98 – 1.00]
Sex, female	220 (47.8)	1 (16.7)	29 (43.9)	3.92 [0.43 – 35.42]
Delivery, C-section	100 (21.7)	0	13 (19.7)	13.1*10^6 [0.0 – Inf]
ReSViNET score [IQR]	3 [2 – 5]	6 [4 – 7]	3 [4 – 6]	0.80 [0.52 – 1.24]
Medication	196 (45%)	4 (67%)	36 (55%)	
Acetaminophen	91 (21%)	3 (50%)	19 (29%)	0.39 [0.04 – 4.13]
Nasal	159 (36%)	1 (17%)	32 (49%)	**24.75 [2.05 – 298.43]**
Salbutamol	19 (4%)	0 (0%)	7 (11%)	NA
Antibiotics	8 (2%)	1 (17%)	0 (0%)	NA
Fever (>= 38.2C)	106* (27.2)	2 (50.0)	25 (42.4)	0.76 [0.10 – 5.80]
Breathing difficulty	221* (56.8)	1 (25.0)	50 (84.7)	**15.0 [1.41 – 159.29]**
Medical attendance	107 (24%)	1 (17%)	35 (54%)	5.65 [0.63 – 50.99]
Hospitalization	8 (2%)	0 (0%)	4 (6%)	NA
ER	7 (2%)	0 (0%)	5 (8%)	NA
GP out-of-hour	30 (7%)	0 (0%)	16 (25%)	NA
GP	84 (19%)	1 (17%)	27 (42%)	NA

Data was obtained by case report forms and episode questionnaires. 458 CRFs were completed, 441 episode questionnaires were completed including information on medication use and medical attendance. Breathing difficulty was defined as chest retractions, nasal flaring, stridor, head bobbing or dyspnea. Acetaminophen also included the use of ibuprofen. Nasal included saline and/or xylometazoline spray. Antibiotics includes the use of any antibiotics. * = data available for 389 ARTIs. ARTI: acute respiratory tract infection; ER: emergency room; GP: general practitioner; RSV: respiratory syncytial virus.

For the hospital cohort, age at admission was not significantly different (76 vs. 68 days, Table 3). Antibiotics were less frequently administered (OR: 0.29, 95% 0.09 – 0.91) and, length of hospital stay was significantly longer for RSV compared with influenza (median 4 days versus 2 days, OR: 1.47). Hospitalized infants tested positive for RSV needed more often respiratory support (OR: 6.32, 95% 1.42 – 28.00), underwent less blood examination (OR: 0.13, 95% 0.05 - 0.34) and had less often fever (>= 38.2 degrees Celsius; OR: 0.18, 95% 0.07 - 0.49) when compared with infants with influenza. One infant with RSV was transferred to the pediatric intensive care unit, none of the influenza-positive infants.

Table 3. Clinical characteristics of hospital cohort. Breathing difficulty was defined as chest retractions, nasal flaring, stridor, head bobbing or dyspnea. No abnormalities were defined as there were no signs of breathing difficulty and no respiratory abnormalities were found during physical examination.

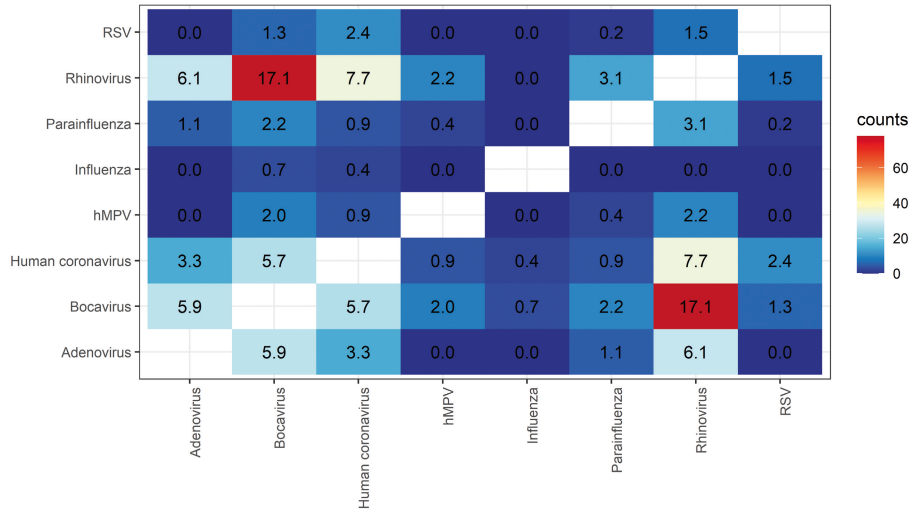
	Total (n=324)	Influenza-ARTI (n=22)	RSV-ARTI (n=154)	OR [95% CI]
Patient characteristics				
Age, median days	71 [36 - 163]	76 [49 - 228]	68 [38 - 138]	1.00 [0.99 – 1.00]
Sex, male	186 (55.0)	14 (63.6)	79 (51.3)	0.60 [0.24 - 1.52]
Delivery, C-section	55 (23.5)	6 (40.0)	20 (19.6)	0.37 [0.12 - 1.16]
Clinical presentation				
Days of complaints before admission	4 [2 - 7]	1 [1 - 5]	4 [3 - 7]	1.05 [0.96 - 1.14]
Fever (>= 38.2C)	116 (34.3)	16 (72.7)	50 (32.3)	**0.18 [0.07 - 0.49]**
Low saturation (<95%)	37 (11.0)	1 (4.5)	23 (14.9)	3.69 [0.47 - 28.76]
Breathing difficulty	120 (35.5)	4 (18.2)	70 (45.5)	**3.75 [1.21 – 11.6]**
No abnormalities	61 (27.5)	11 (68.8)	19 (17.6)	**0.10 [0.03 – 0.31]**
Patient management				
Respiratory support	94 (27.8)	3 (13.0)	60 (38.7)	**6.21 [1.40 - 27.54]**
Antibiotics	44 (13.0)	5 (21.7)	12 (7.7)	**0.29 [0.09 - 0.91]**
Patient outcome				
Length of stay	3 [2 - 5]	2 [1 - 3]	4 [2 -5]	**1.47 [1.11 - 1.95]**

Other viruses

Suppl Figure 2 shows viruses detected per age group for the birth cohort, which shows an significant increase of detection rate with increasing age (GEE: p = 0.018). For the birth cohort, in almost half of the positive samples two or more pathogens were identified (48.0%, 202/421; Suppl Table 1). Rhinovirus was the most commonly detected virus (67.8%, 137/202; Figure 1A) in coinfections, followed by bocavirus (59.4%; 120/202). RSV was the only pathogen more often detected solely. Influenza was not significantly detected more often in coinfection. Most common combination in hospitalized infants overall was RSV and rhinovirus in 3.4% (11/324), followed by RSV and coronavirus with 3.1% (10/324; Figure 1B). For the birth cohort the most common combination was rhinovirus and bocavirus with 17.1% (78/457), followed by rhinovirus and human coronavirus (7.7%; 35/457). Influenza was mostly seen together with bocavirus in both birth and hospital cohort, in 50.0% (3/6) and 9.7% (2/23) of the influenza cases, respectively. In the hospital cohort, RSV was mostly detected

with rhinovirus (7.3%; 11/150). For the birth cohort this was human coronavirus with RSV (16.4%; 11/67).

A.



B.

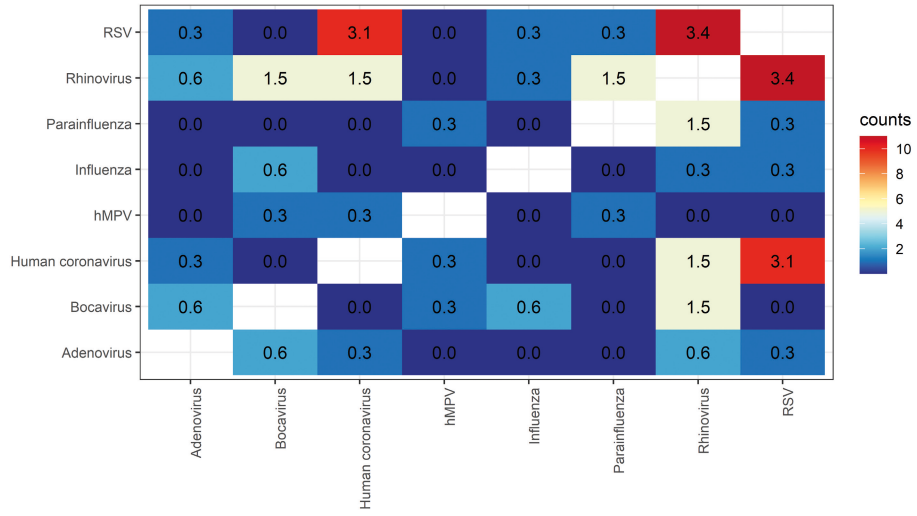


Figure 1. Coinfections in both cohorts
Coinfection in both cohorts, shown separately. Number in columns shows percentage of incidence coinfection of all taken swabs, color of the column shows the absolute incidence of coinfection. hMPV: human metapneumovirus; RSV: respiratory syncytial virus.
(A) birth cohort (n=457). (B) hospital cohort (n=324)

DISCUSSION

In our combined prospective study of previously healthy term infants an incidence of 35.8% of RSV-ARTI and 3.2% of influenza-ARTI was found during three consecutive and corresponding RSV seasons. RSV-ARTI resulted significantly more in medical consultation compared to influenza-ARTI. For the infants hospitalized due to an ARTI between October and April, RSV was the most commonly detected virus (46.3%), followed by rhinovirus (24.4%) and influenza (7.1%). Infants younger than three months of age had the highest risk of being hospitalized due to RSV, for influenza this was throughout their first year of life.

Incidence of RSV MA-ARTI in our birth cohort was higher compared to other cohort studies. We found an incidence of 19.3% in all participants, where previous studies found an incidence of 8 to 12% [3,9,10,16]. This could be due to a higher endemicity during our cohort or high percentage of daycare attendance in our cohort. A meta-analysis estimated an incidence of 5.2% for influenza-ARTIs and 1.5% influenza-associated ALRI in infants from developed countries [8]. We found an incidence of 16.7% for MA-ARTIs due to influenza, however this had a wide confidence interval. Regarding the proportion of influenza viruses in hospitalized infants, previous studies showed a percentage of 5.0 to 6.1% of the admissions [6,7,19,20], we found a percentage of 7.1%. For RSV, two large cohorts by Hall et al. showed that RSV was associated with 27.4% [21] and 24.0% [9] of the hospitalizations in infants with respiratory infections. These findings compared to our results show a higher burden for RSV in hospitalized infants [22]. Our results on incidence of both viruses related to age are comparable to similar studies [23,24]. Incidence of RSV hospitalization is highest early in infancy, with a peak during the second month of life. For influenza there was only a minor peak during early infancy [23,24]. Due to low influenza cases, we were not able to analyze clinical characteristics for influenza-ARTI in our birth cohort. Analysis of clinical characteristics in our hospital cohort showed that fever occurred more often in influenza-ARTI, also antibiotics were more prescribed and ancillary testing was more often performed. The higher rate of fever is consistent with previous reports [22,25]. For RSV-ARTI, symptoms on breathing difficulty were more often found and consequently infants needed more often respiratory support. Conversely, almost 70% of influenza-ARTI did not show any respiratory symptoms. Length of stay was significantly longer for RSV-ARTI compared with influenza, which has also previously been described [26,27]. In our study specific

symptoms on coughing or wheezing during admission were not specifically reported, however literature showed predominance of cough over fever in RSV infected infants. Influenza caused a significantly higher rate of fever than infants with RSV infection [22,25].

Because of the presented results the need for protective strategies is warranted. With the highest incidence of ARTIs and hospitalizations due to RSV, especially in young infants. With influenza vaccination in pregnant women advised and upcoming preventive and therapeutic interventions for RSV coming, infants can be protected early in life against these pathogens. According to our findings, the need for early life protection against respiratory viruses should be focused on RSV. However, our data is based on a cohort in the Netherlands for healthy term born infants. About 82% of the in-hospital deaths due to influenza occurred in low-income and lower-middle-income countries, showing that preventive and therapeutic strategies are most needed in these countries [28]. In addition, pregnant women have higher risk of hospitalization due to influenza compared to non-pregnant women [29]. Maternal influenza vaccination protects pregnant women [30], while RSV has no substantial burden in healthy pregnant women. It is estimated that maternal vaccination against both pathogens could have a substantial impact on decreasing life-threatening infections in infants [31,32].

A strength of this study is that we used a community-based birth cohort in healthy term born infants, which were followed intensively throughout their first year of life. A respiratory swab was taken at the time of any symptoms of an ARTI, without the need of medical attendance, excluding selection bias for viral testing based on disease severity. During the RSV season (which includes the influenza season) 89% of the participants were swabbed within five days after onset of ARTI symptoms. Both RSV and influenza were endemic in all included seasons. To provide more robust information on severity of both pathogens, we used a birth and hospital cohort. Both hospital and birth cohort were executed during the same RSV seasons and in the same area, ruling out differences in yearly endemicity of influenza and RSV between the cohorts. In addition, performed viral analysis was similar in both cohorts.

There are several limitations to our study. First, sample collection was based on respiratory symptoms. Especially influenza did not always result in respiratory symptoms in infants. This could have caused selection bias and therefore an

underestimation of influenza incidence. Thompson et al. showed recently when focusing on respiratory diagnoses influenza-associated hospital admissions among infants was underestimated [20]. This could be one of the reasons for the low number of influenza cases in our study, leading to an underestimation of influenza incidence. Second, our birth cohort was based on a Dutch population, mainly living in an urban area and a high percentage of infants visiting daycare, increasing the risk of exposure to respiratory viruses and infections. This could have led to an overestimation of the incidence. Third, data on hospitalized infants was retrieved retrospectively, therefore not all variables were documented consistently or important data was lacking, for example whether they were breastfed or if there was daycare attendance. Last, viral testing was qualitative and did, therefore, not provide viral load, however the aim of the paper focused on burden of influenza and RSV.

In conclusion, we showed with our birth and hospital-based cohorts of healthy term born infants a relative low incidence for influenza compared to RSV. There were around eleven times more RSV ARTIs than influenza ARTIs in the birth cohort and around seven times more hospitalizations due to RSV compared with influenza. This high burden of RSV highlights the need of preventive and therapeutic strategies.

Notes

Study group members

The RESCEU investigators are as follows:

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Conflict of interests

All remaining authors declare no competing interests.

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REFERENCES

1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet [Internet]. **2016** [cited 2019 Mar 21]; 388(10063):3027–3035. Available from: http://tinyurl.com/Hopkins-
2. Troeger C, Forouzanfar M, Rao PC, et al. Articles Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis [Internet]. **2017** [cited 2021 Jan 8]; 17:1133–1161. Available from: http://dx.doi.org/10.1016/
3. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet [Internet]. Elsevier Ltd; **2010**; 375(9725):1545–1555. Available from: http://dx.doi.org/10.1016/S0140-6736(10)60206-1
4. Acute lower respiratory infections. Eur. Lung Found. . 2019.
5. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet [Internet]. **2017** [cited 2018 Jun 25]; 390(10098):946–958. Available from: https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(17)30938-8.pdf
6. Wang X, Li Y, O'Brien KL, et al. Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. Lancet Glob Heal [Internet]. Elsevier Ltd; **2020** [cited 2021 Jun 14]; 8(4):e497–e510. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214109X19305455
7. Lafond KE, Nair H, Rasooly MH, et al. Global Role and Burden of Influenza in Pediatric Respiratory Hospitalizations, 1982–2012: A Systematic Analysis. Tumwine JK, editor. PLOS Med [Internet]. A Systematic Analysis; **2016** [cited 2019 Oct 22]; 13(3):e1001977. Available from: https://dx.plos.org/10.1371/journal.pmed.1001977
8. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to seasonal influenza in young children: A systematic review and meta-analysis. Lancet [Internet]. Lancet; **2011** [cited 2021 Jan 19]; 378(9807):1917–1930. Available from: http://soar.wichita.edu/dspace/handle/10057/646
9. Hall CB, Weinberg GA, Iwane MK, et al. The Burden of Respiratory Syncytial Virus Infection in Young Children. N Engl J Med [Internet]. **2009** [cited 2018 Jun 13]; 360(6):588–598. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa0804877
10. Zomer-Kooijker K, Uiterwaal CSPM, Gugten AC Van Der, Wilbrink B, Bont LJ, Ent CK Van Der. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. Eur Respir J [Internet]. **2014** [cited 2018 Jun 13]; 44(3):666–674. Available from: http://ow.ly/xlkcL
11. Anderson LJ, Dormitzer PR, Nokes DJ, Rappuoli R, Roca A, Graham BS. Strategic priorities for respiratory syncytial virus (RSV) vaccine development. Vaccine [Internet]. **2013**; 31 Suppl 2:B209-15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23598484

12. WHO. Vaccines against influenza WHO position paper – November 2012. Wkly Epidemiol Rec [Internet]. **2012** [cited 2021 Jun 29]; 87(47):461–76. Available from: http://web.a.ebscohost.com.proxy.library.uu.nl/ehost/pdfviewer/pdfviewer?vid=1&sid=6e9a3ad6-a1b7-43b0-b154-4751cc3138e6%40sessionmgr4006
13. Madhi SA, Polack FP, Piedra PA, et al. Respiratory Syncytial Virus Vaccination during Pregnancy and Effects in Infants. N Engl J Med [Internet]. Massachusetts Medical Society; **2020** [cited 2021 Jan 21]; 383(5):426–439. Available from: https://pubmed.ncbi.nlm.nih.gov/32726529/
14. Mazur NI, Higgins D, Nunes MC, et al. The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates [Internet]. Lancet Infect. Dis. 2018 [cited 2018 Jun 25]. p. e295–e311. Available from: www.thelancet.com/infection
15. Navarro Alonso JA, Bont LJ, Bozzola E, et al. RSV: perspectives to strengthen the need for protection in all infants. Emerg Themes Epidemiol [Internet]. BioMed Central Ltd; **2021** [cited 2022 Sep 19]; 18(1):1–5. Available from: https://ete-online.biomedcentral.com/articles/10.1186/s12982-021-00104-5
16. Wildenbeest JG, Zuurbier RP, Korsten K, et al. Respiratory Syncytial Virus Consortium in Europe (RESCEU) Birth Cohort Study: Defining the Burden of Infant Respiratory Syncytial Virus Disease in Europe. J Infect Dis [Internet]. **2020** [cited 2020 Sep 16]; . Available from: https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa310/5892596
17. Wildenbeest JG, Billard M-N, Zuurbier RP, et al. The burden of respiratory syncytial virus in healthy term-born infants in Europe: a prospective birth cohort study. Lancet Respir Med [Internet]. **2022**; 2600(22):1–13. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213260022004143
18. Justicia-Grande AJ, Pardo-Seco J, Cebey-López M, et al. Development and Validation of a New Clinical Scale for Infants with Acute Respiratory Infection: The ReSVinet Scale. Esposito S, editor. PLoS One [Internet]. Public Library of Science; **2016** [cited 2019 Feb 12]; 11(6):e0157665. Available from: https://dx.plos.org/10.1371/journal.pone.0157665
19. Poehling KA, Edwards KM, Griffin MR, et al. The Burden of Influenza in Young Children, 2004-2009. Pediatrics [Internet]. **2013** [cited 2021 Jan 19]; 131(2):207–216. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2012-1255
20. Thompson MG, Levine MZ, Bino S, et al. Underdetection of laboratory-confirmed influenza-associated hospital admissions among infants: a multicentre, prospective study. **2019** [cited 2021 Jun 17]; . Available from: http://dx.doi.org/10.1016/
21. Hall CB, Weinberg GA, Blumkin AK, et al. Respiratory Syncytial Virus-Associated Hospitalizations Among Children Less Than 24 Months of Age. Pediatrics [Internet]. **2013**; 132(2):e341–e348. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2013-0303
22. Amini R, Gilca R, Boucher FD, Charest H, Serres G De. Respiratory syncytial virus contributes to more severe respiratory morbidity than influenza in children < 2 years during seasonal influenza peaks. Infection [Internet]. **2019** [cited 2019 Sep 9]; 47(4):595–601. Available from: https://link-springer-com.proxy.library.uu.nl/content/pdf/10.1007%2Fs15010-019-01287-5.pdf
23. Jensen A, A. F. Simões E, Bohn Christiansen C, Graff Stensballe L. Respiratory syncytial virus and influenza hospitalizations in Danish children 2010–2016. Vaccine [Internet]. **2021** [cited 2021 Jun 14]; 39(30):4126–4134. Available from: https://doi.org/10.1016/j.vaccine.2021.05.097

24. Matias G, Taylor R, Haguinet F, Schuck-Paim C, Lustig R, Shinde V. Estimates of hospitalization attributable to influenza and RSV in the US during 1997–2009, by age and risk status. BMC Public Health [Internet]. **2017** [cited 2020 Dec 4]; 17(1):271. Available from: http://www.hcup-us.ahrq.gov/nisoverview.jsp.
25. Ma X, Conrad T, Alchikh M, Reiche J, Schweiger B, Rath B. Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to systematic literature review. Rev Med Virol [Internet]. Wiley-Blackwell; **2018** [cited 2018 Jul 30]; :e1997. Available from: http://doi.wiley.com/10.1002/rmv.1997
26. Forster J, Ihorst G, Rieger CHL, et al. Prospective population-based study of viral lower respiratory tract infections in children under 3 years of age (the PRI.DE study). Eur J Pediatr [Internet]. **2004**; 163(12):709–716. Available from: http://link.springer.com/10.1007/s00431-004-1523-9
27. Resch B, Eibisberger M, Morris N, Müller W. Respiratory Syncytial Virus- and Influenza Virus-associated Hospitalizations in Infants Less Than 12 Months of Age. Pediatr Infect Dis J [Internet]. **2011**; 30(9):797–799. Available from: https://journals.lww.com/00006454-201109000-00019
28. Wang X, Li Y, O'Brien KL, et al. Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. Lancet Glob Heal [Internet]. **2020** [cited 2021 Jan 8]; 8(4):e497–e510. Available from: www.thelancet.com/lancetgh
29. Mazagatos C, Delgado-Sanz C, Oliva J, Gherasim A, Larrauri A. Exploring the risk of severe outcomes and the role of seasonal influenza vaccination in pregnant women hospitalized with confirmed influenza, Spain, 2010/11-2015/16. PLoS One [Internet]. PLoS One; **2018** [cited 2022 Feb 22]; 13(8). Available from: https://pubmed.ncbi.nlm.nih.gov/30089148/
30. Madhi SA, Cutland CL, Kuwanda L, et al. Influenza Vaccination of Pregnant Women and Protection of Their Infants. N Engl J Med [Internet]. Massachusetts Medical Society; **2014** [cited 2021 Jun 28]; 371(10):918–931. Available from: https://www-nejm-org.proxy.library.uu.nl/doi/10.1056/NEJMoa1401480
31. Löwensteyn YN, Nair H, Nunes MC, et al. Estimated impact of maternal vaccination on global paediatric influenza-related in-hospital mortality: A retrospective case series. EClinicalMedicine [Internet]. **2021** [cited 2021 Jun 15]; :100945. Available from: http://creativecommons.org/licenses/by/4.0/
32. Scheltema NM, Kavelaars XM, Thorburn K, et al. Potential impact of maternal vaccination on life-threatening respiratory syncytial virus infection during infancy. Vaccine [Internet]. The Author(s); **2018**; . Available from: https://doi.org/10.1016/j.vaccine.2018.06.021

SUPPLEMENTARY DATA

Suppl Table 1. Incidence respiratory viruses in both cohorts.

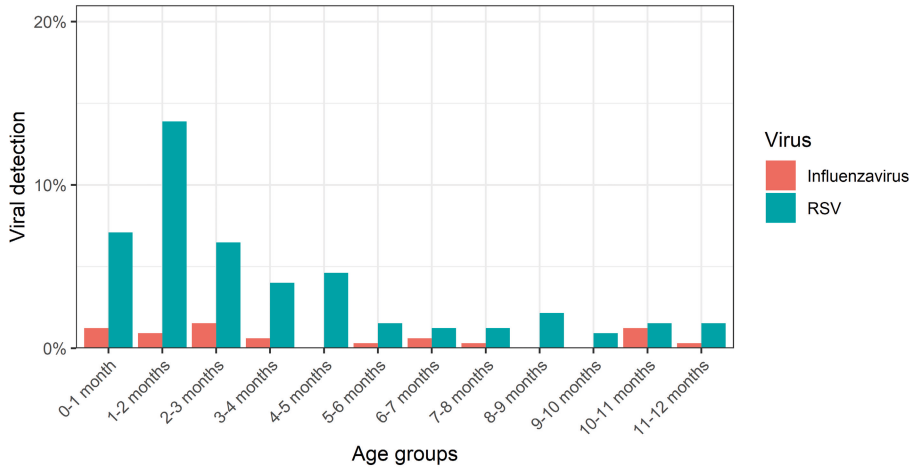
	Hospital cohort				Birth cohort			
	All (n=324)	**Single (n=228)**	**Coinfection (n=42)**	**p-test**	**All (n=457)**	**Single (n=219)**	**Coinfection (n=202)**	**p-test**
Any virus	270 (83.3%)	-	-	-	421 (92.1%)	-	-	-
Adenovirus	11 (3.4%)	6 (2.6)	5 (11.9)	**0.018**	59 (12.9%)	7 (3.2%)	52 (25.7%)	**<0.001**
Bocavirus	10 (3.1%)	1 (0.4)	9 (21.4)	**<0.001**	133 (29.1%)	13 (5.9%)	120 (59.4%)	**<0.001**
hMPV	13 (4.0%)	10 (4.7)	3 (7.1)	0.708	22 (4.8%)	5 (2.3%)	17 (8.4%)	**0.009**
Rhinovirus	79 (24.4%)	54 (23.7)	25 (59.5)	**<0.001**	250 (54.7%)	113 (51.6%)	137 (67.8%)	**0.001**
PIV	13 (4.0%)	6 (2.6)	7 (16.7)	**<0.001**	32 (7.0%)	8 (3.7%)	24 (11.9%)	**0.003**
Human coronavirus	17 (5.2%)	2 (0.9)	15 (35.7)	**<0.001**	94 (20.6%)	22 (10.0%)	72 (35.6%)	**<0.001**
Influenza	23 (7.1%)	20 (8.8)	3 (7.1)	0.963	6 (1.3%)	2 (0.9%)	4 (2.0%)	0.609
RSV	150 (46.3%)	129 (56.6)	21 (50.0)	0.536	71 (15.5%)	49 (22.4%)	22 (10.9%)	**0.003**

Table shows number of viruses detected per cohort (hospital and birth cohort) and divided into single detection or as coinfection. P values were calculated with chi-square test. hMPV: human metapneumovirus; PIV: parainfluenza virus; RSV: respiratory syncytial virus.

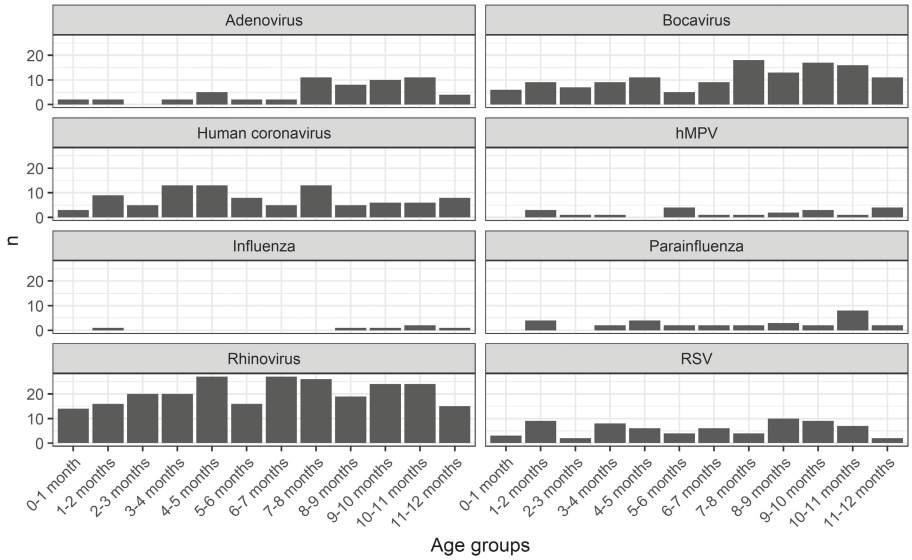
Suppl Table 2. Clinical symptoms of respiratory episodes in birth cohort.

	Total (n=389)	Influenza-ARTI (n=4)	RSV-ARTI (n=59)
Rhinitis (%)	383 (98%)	3 (75%)	58 (98%)
Coughing (%)	347 (89%)	4 (100%)	59 (100%)
Wheezing (%)	199 (51%)	0 (0%)	51 (86%)
Dyspnea (%)	221 (57%)	1 (25%)	50 (85%)
Apnea (%)	21 (5%)	0 (0%)	5 (9%)
Feeding intolerance (%)	230 (59%)	2 (50%)	50 (85%)
Vomiting (%)	154 (40%)	1 (25%)	34 (58%)
Fever (>= 38,2) (%)	106 (27%)	2 (50%)	25 (42%)
Duration of symptoms, days (median [IQR])	12 [7 – 14]	11 [7 – 13]	12 [9 – 14]
Peak of symptoms, days (median [IQR])	4 [2 – 8]	4 [3 – 4]	4 [3 – 6]
Health of child [0 – 100] (median [IQR])	60 [50-69]	56 [51-63]	50 [43-59]

Data on symptoms were obtained by parental diaries of 389 episodes. Duration of symptoms were calculated by number of diaries completed for symptoms, with a maximum of 14 days. Health of child was calculated by the worst score parents gave, the scale ranged from 0 to 100, with 100 as best score. Peak of symptoms was calculated by the day with the worst score on health of child scale. ARTI: acute respiratory tract infection; RSV: respiratory syncytial virus.

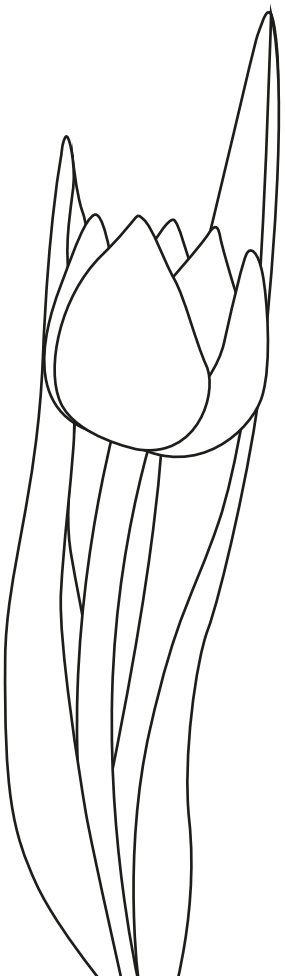


Suppl Figure 1. Age at admission of influenza and RSV ARTI in hospital cohort
X-axis shows age per month. Y-axis shows the percentage of viral detection per virus (n=324). Color shows type of virus: influenza or RSV.
RSV: respiratory syncytial virus.



Suppl Figure 2. Age distribution per virus in birth cohort
X-axis shows age per month. Y-axis shows the number of viruses detected (n=457), divided into columns per virus.
hMPV: human metapneumovirus; RSV: respiratory syncytial virus.

You can check out any time you'd like
But you can never leave
(The Eagles – Hotel California)



Chapter 8

General discussion

SUMMARY OF THE MAIN FINDINGS OF THIS THESIS

In this thesis I have described the use of different point-of-care tests (POCTs) on respiratory syncytial virus (RSV) as well as the burden and dynamics of respiratory viruses in infants. I will first summarise the main findings of this thesis after which I will discuss clinical implications and future perspectives of part one on RSV point-of-care testing.

1. Part one: RSV point-of-care testing

In **part one** of the thesis I described the use of different POCTs on RSV in infants and in older adults. In recent years, several POCTs have been developed to detect RSV. Our studies were performed within our international RESCEU birth cohort and older adult cohort in which the primary objective was to determine the burden of RSV. Because of the prospective character of these cohort studies we were able to examine the accuracy of different rapid tests in a community setting, with different levels of severity of RSV infections.

In **Chapter 2** the performance of rapid antigen detection test BinaxNOW® RSV (BN) was evaluated. Samples of infants with acute respiratory tract infections (ARTIs) with different degrees of disease severity were analysed with BN compared to molecular diagnosis. In total, 162 respiratory samples from 148 children were studied. Low sensitivity was found of rapid antigen test BN for RSV detection in infants, with a sensitivity of 7.6% (95% CI 3.3-16.5%), specificity was 100% (95% CI 96.2-100%). Sensitivity was slightly higher in the subgroup of infants admitted to a PICU compared to less severe ill infants (22.2% versus 5.3%), although this difference was not statistically significant (p=0.134) and sensitivity remained low.

The performance of a molecular rapid detection test in older adults was assessed in **Chapter 3.** Respiratory samples of participants of RESCEU's older cohort study were collected each time they experienced an ARTI. Performance of the Xpert® Xpress Flu/RSV assay was evaluated to diagnose RSV infection in home-dwelling older adults (≥60 years) with ARTI in different clinical settings. The performance of Xpert® Xpress Flu/RSV compared to routine RT-PCR is high for RSV detection in home-dwelling older adults. In all cases with discordant results for the two assays, viral load was low. The positive percentage agreement (PPA) was 90.9% (95% CI 76.4-96.8%) and negative percentage agreement (NPA) was 99.7% (95% CI 99.0-99.9%). PPA was used

as outcome, rather than sensitivity, to show agreement between two accurate tests. The assay is fast and easy to use and therefore has the ability to improve patient management and outcomes.

2. Part two: respiratory viruses in infants

In **part two** of this thesis I discussed the burden and dynamics of respiratory viruses in infants. Results are based on a hospital based cohort and two different birth cohort studies: the Dutch MUIS birth cohort study and the international RESCEU infant cohort study. Both birth cohort studies, MUIS and the Dutch part of RESCEU, were performed at a general hospital, Spaarne Gasthuis in the northern part of the Netherlands. Within these cohorts we were able to investigate different degrees of severity.

In **Chapter 4** we examined the occurrence of respiratory viruses in infants during the first year of life. 1,304 nasopharyngeal samples were obtained from 11 consecutive regular sampling moments and during an ARTI. Rhinovirus (RV) was negatively associated with ARTI (aOR 0.41 [95% CI 0.18-0.92]). Human metapneumovirus, RSV, parainfluenza (PIV) 2 and 4, and human coronavirus (HCoV) HKU1 were positively associated with ARTI. Asymptomatic RV in early life was, however, associated with increased susceptibility to and recurrence of ARTIs later in the first year of life (Kaplan-Meier survival analysis: p=0.022). Overall, respiratory viruses are often detected in infants and are often asymptomatic.

The methods of the prospective international RESCEU birth cohort study was described in **Chapter 5**. This multicenter study had the aim to recruit 10,000 healthy term infants during 3 consecutive years, including a nested cohort of 1,000 infants who were followed actively. In this nested cohort, during all ARTIs in the RSV season, a respiratory swab was collected for RSV molecular diagnosis. The primary outcome was the incidence of RSV associated ARTI, medically attended (MA)-ARTI, and hospitalisation in the first year of life. This will provide key information to fill the gaps in knowledge about the burden of RSV disease in healthy term infants and support decision making for implementation of new prevention strategies.

The results of this study were shown in **Chapter 6**. In total 9,154 infants born between July 2017 and April 2020 were followed during the first year of life of whom 993 participated in the nested active surveillance. The incidence of RSV hospitalisation

in the total cohort was 1.8% (95% CI 1.6-2.1). About half of hospitalisations for respiratory tract infection in the first year of life were associated with RSV. The majority (57.9%) of RSV hospitalizations occurred in children <3 months of age. Incidences of RSV infection and medically-attended RSV infection in the active surveillance cohort were 26.2% (95% CI 24.0-28.6) and 14.1% (95% CI 12.3-16.0), respectively. Immunisation of pregnant women or healthy term-born infants during their first winter season could have a significant impact on the healthcare burden caused by RSV infections.

The burden of influenza and RSV in infants of the Dutch part of RESCEU was discussed in **Chapter 7**. An overall incidence of 3.2% (6/187) for influenza-ARTI and 35.8% (67/187) for RSV-ARTI was found in the birth cohort. The hospital cohort was performed in a general hospital, Spaarne Gasthuis. In this cohort, influenza was detected in 7.6% (23/304) of hospitalised infants with an ARTI, for RSV this was 49.3% (150/304). RSV was responsible for the highest number of ARTIs in both non-hospitalised and hospitalised infants, especially during the first months of life. Incidence of influenza-ARTI was low compared to RSV-ARTI. These findings suggest most emphasis should be on RSV prevention strategies, especially in the first months of life.

GENERAL DISCUSSION

1.RSV diagnostics

Since 1956, when respiratory syncytial virus (RSV) was isolated for the first time by cell culture, it became clear that RSV is a major cause of respiratory tract infections in infants leading to a significant burden worldwide. It was estimated that RSV was associated with 33.0 million cases of LRTI, responsible for 3.6 million LRTI hospitalisations and 101,400 RSV-attributable overall deaths in children <5 years in 2019 worldwide per year [1]. In addition, older adults (≥ 65 years of age) also have a higher risk for more complicated course of the infection. This high-risk group has an estimated burden ranging from 3% to 7% [2,3]. Treatment options are limited, mostly only supportive care is available for patients with severe RSV infection. Consequently, vaccine development for RSV focuses on high-risk groups and the end of the age spectrum: the very young and older population. Recently, several phase 3 trials have been initiated in older adults and pregnant women (Novavax [4,5], Pfizer [6]). In addition, seasonal prophylaxis with extended half-life RSV-specific antibodies showed promising results in late preterm infants (Nirsevimab [7]). Despite considerable efforts to develop RSV antivirals [8], this has not yet led to a market-approved antiviral therapy.

The gold standard for RSV diagnosis was previously cell culture. Isolation of RSV in tissue culture requires technical expertise and appropriate specimen handling to maintain viral viability. Turnaround time for culture is relatively long and varies between 2 to 7 days. The advantage of cell culture is that it detects exclusively infectious virus and that it can also detect new viral strains. Despite its excellent specificity, sensitivity is relatively poor and estimated at 46% compared to other techniques as reverse transcriptase polymerase chain reaction (RT-PCR)[9].

Laboratory based RT-PCR has become the gold standard. This technique has been invented in 1983 and uses thermal cycling for nucleic acid amplification (NAA). Specific primers for the pathogen of interest and DNA polymerase are added and if the pathogen is present in the sample, it generates multiple copies which leads to a detectable signal. The sensitivity is high and specificity is comparable with cell culture[10]. In contrast to cell culture PCR detects viral RNA or DNA and does not give information whether the detected virus is still viable/infectious. In addition, this technique only allows detection of already known virus strains, making this technique

unsuitable for detection of new viruses or strains. RT-PCR relies on trained laboratory staff and specialised equipment, and it normally takes 24-48 hours to provide results. Moreover, multiplex PCR is able to detect multiple targets at once, like over 20 respiratory pathogens. Another laboratory based technique is immunofluorescence. Sensitivity comparable with cell culture, but this technique is based on microscopic detection and therefore needs expertise and is labor-intensive [10].

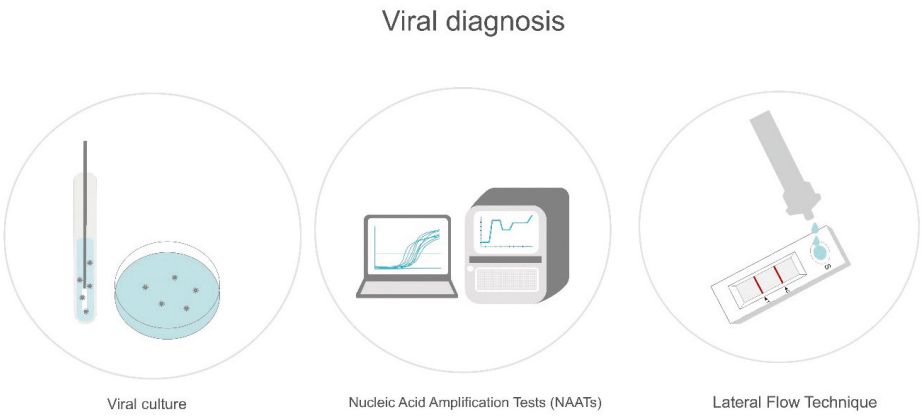


Figure 1. Overview of different viral diagnostic tests

For the development and use of novel RSV antivirals [11] and evaluation of efficacy of new RSV vaccines, there is an evolving role for rapid tests to detect RSV within several minutes as a companion diagnostic. In a recent phase 2 antiviral trial [12] it was suggested that antivirals should be administered in an early stage of disease, before the development of a lower respiratory tract infection. This leaves a narrow time window to confirm RSV as the causative pathogen. Therefore, there is a need for reliable and rapid RSV point-of-care tests in a community-based setting to enable to start therapy promptly after start of symptoms. The sensitivity and time to diagnosis of RSV is improved substantially from cell culture to the recently developed molecular-based point-of-care tests, which are easy to use and typically show results within 15 minutes to 2 hours. Reliable rapid diagnostic tests can also help to enable cohorting of hospitalised patients in the RSV season, to prevent nosocomial infections, and to improve patient management regarding policies such as giving unnecessary use of antibiotics. As described in a Dutch study, approximately one-third of hospitalised children with RSV were unnecessarily treated with antibiotics [13]. Antibiotics were prescribed in case of severe symptoms or if the diagnosis of RSV was not clear at that moment. Rapid diagnosis of RSV could therefore possibly

lower overuse of antibiotics in RSV-infected patients. However, a study showed that rapid diagnosis of respiratory viruses in hospitalised adults with LRTI did not reduce antibiotic use or costs [14]. This could be due to the fact that most antibiotics were already prescribed by the general practitioner and those not responding to treatment of with severe clinical presentation were referred to the hospital. But also because clinical management hardly changed with fast diagnostic tests, showing clinical decision making is an important factor in reducing antibiotic use [14].

2. Rapid antigen detection tests

In the need for rapid diagnosis of respiratory pathogens bedside diagnostic tests have been developed, the so called point-of-care tests (POCTs). In 2002, the first RSV POCT was developed: a rapid antigen detection test (RADT). RADTs are easy to use by non-laboratory personnel and have a turnaround time of approximately 15 minutes. They are often less expensive compared to routine RT-PCR assays. The most commonly used technique is lateral flow immunographic assay, like the BinaxNOW RSV (Alere Inc., Waltham, MA) [15]. In terms of technique, these tests are comparable with a pregnancy test and are disposable. The diluted specimen is added to the test strip and starts migrating from the sample port to the result and control line (Figure 2). Virus-specific antibodies in the sample port can bind to the specimen. On the test line it binds a second set of vires-specific antibodies. This binding produces the appearance of a coloured line and shows a positive result. A positive control line indicates a valid test.

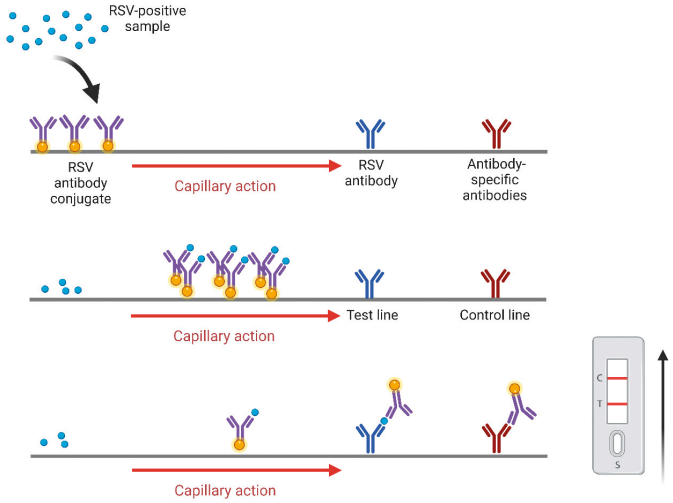


Figure 2. Lateral flow immunographic technique
RSV = respiratory syncytial virus. Created with BioRender.com

The advantage of RADTs is that they are fast and easy to use and no ancillary equipment is necessary. In addition, no expertise is needed to use these test, making it possible to be used in the community and on a massive scale.

These RADTs have a high specificity, although a wide range in sensitivity, generally depending on viral load, which is related to age and disease severity [16,17], resulting in a higher sensitivity in children compared to adults and in severe disease [18]. Two recent meta-analyses, including all ages, showed a pooled sensitivity of 74% (95% CI, 71% to 78%) [19] and 70.9% (95% CI, 63.0% to 77.8%) [20] for RSV RADTs compared with RT-PCR. However, these studies bear the risk of overestimating test accuracy as they were performed in medically attended or hospitalised patients, used remnant specimens, were partially performed in patients with predictable high viral loads, were mostly sponsored by the manufacturer, and were performed in relatively small numbers of patients. Many studies are performed retrospectively and in hospitalised children, while the tests are not evaluated at point-of-care or in patients with mild disease. As a result, sensitivity of individual studies vary considerably from 41.2% [21] to 83% [22]. In **Chapter 2** of this thesis, we prospectively evaluated the performance of a RADT BinaxNOW RSV in infants with ARTI. We showed a very low sensitivity of 7.6% in symptomatic hospitalised infants and infants tested at home [23]. This is remarkable as sensitivity was expected to be higher, especially in infants because of a suspected higher viral load. Even in infants with severe disease in whom viral

load is likely to be the highest, sensitivity was only 22%. Low sensitivity in our study could be explained by sampling methods, as nasal (mid-turbinate) swabs were taken instead of nasopharyngeal swabs or aspirates. However, we do not think sampling methods fully explain the low sensitivity of BN. Another explanation could be temporal evolution of the binding site of the RSV fusion (F) protein. Although, the F-protein is generally well conserved, making it unlikely as an explanation for the low sensitivity. Therefore, POCTs, i.e. molecular, with a higher sensitivity than RADTs are needed for the reliable diagnosis of RSV.

3. Molecular POCTs

Since 2015, PCR-based or molecular POCTs entered the market and are used more often in daily clinical practice. They are also fast, easy to use by non-laboratory personnel, and often less expensive compared to routine RT-PCR. The turnaround time of most molecular POCTs is 15 minutes to one hour. These assays are all based on NAA, using different techniques to make the process almost fully automated, like loop-mediated isothermal amplification (LAMP). Some molecular POCTs also provide semi-quantitative measures (Ct-value). Studies report a high sensitivity and specificity ranging from 86% to 100% and from 93 to 100%, respectively, generally depending on viral load [10]. Moreover, they show more promising performance compared with RADTs. However, studies reporting on accuracy still bear the risk of overestimating test accuracy as they were performed in medically attended or hospitalised patients [24–27], used remnant specimen [24,26], were partially performed in children with predictable high viral loads [25,26], were mostly sponsored by the manufacturer [25–27], and were performed in relatively small numbers of patients [24,26,28].

Examples of low complexity molecular POCTs are: ID NOW™ RSV (Abbott Diagnostics Scarborough, Inc., Scarborough, ME), BioFire FilmArray® Respiratory Panel EZ (bioMérieux, Marcy-l'Étoile, France), cobas® Liat® Influenza A/B and RSV (Roche Diagnostics, Forrenstrasse, Switzerland), and Xpert® Xpress Flu/RSV (Cepheid, Sunnyvale, CA, USA). These tests are also approved for use outside the laboratory in the United States (clinical laboratory improvement amendments (CLIAs)-waived by the Food and Drug Administration (FDA)).

3.1 IDNow™ RSV

The IDNow™ RSV, formerly known as Alere i™, provides qualitative detection of RSV. The technology is based on nicking enzyme amplification reaction (NEAR) [29]. NEAR

makes nucleic amplification possible at constant low temperatures and does not need thermal cycles. The turnaround time (TAT) is 13 minutes, including 3 minutes sample preparation and insert of the cartridge and sample. The assay runs for 10 minutes until RSV is detected, if not the test will show a negative result. The device is small and portable, it can run one sample at the time, which limits high throughput. The cartridge package consists of a sample receiver with a buffer, a test base with the reagents for RSV amplification targeting a unique region of the nonstructural gene NS2 and a transfer pipette. After three minutes of warming the sample receiver, the viral transport medium (VTM) or direct swab is added, then it is transferred to the test base. Further steps are then fully automated by the assay. The assay will amplify the specimen and a positive result will follow if RSV is detected within 10 minutes. In addition, for the assay there are also cartridges available for other pathogens at this moment, like COVID-19, Influenza A & B, and group A streptococcus.

3.2 Xpert® Xpress Flu/RSV

The Xpert® Xpress Flu/RSV is a quantitative assay for RSV detection. The assay is performed on Cepheid GeneXpert Instrument Systems [30]. These systems automate and integrate sample extraction, nucleic acid purification and amplification, and detection of target sequences from clinical specimens by using reverse transcription (conversion of RNA templates into DNA) followed by real-time PCR. The primers and probes target the genes encoding the RSV A and B nucleocapsid (N gene). Each test requires the use of a single-use disposable GeneXpert cartridge that contains target-specific reagents and carries out the PCR processes. Viral transport medium containing the specimen is transferred to the sample chamber of the disposable Xpert® Xpress Flu/RSV Assay cartridge. The assay runs for 40 full PCR cycles and is early terminated if the threshold for a positive test result is reached. Test results are obtained in approximately 30 minutes. The GeneXpert reports, if the test is positive, the semi-quantitative measure of Ct-value. GeneXpert can also be used for multiple other pathogens, like bacterial pathogens, SARS-CoV-2, influenza and pathogens combined like Xpert® Xpress CoV-2/Flu/RSV. Besides, GeneXpert systems are available in a two, four, 16, 48, or 80-module configuration, making it possible to run multiple tests at one time and therefore making high throughput possible.

Molecular-based POCTs are highly specific, ranging from 93 to 100% [10]. Sensitivity is in general higher compared to RADTs, ranging from 87 to 100% [10]. In **Chapter 3** we showed in our prospective community cohort consisting of older adults an

excellent performance of Xpert® Xpress Flu/RSV. In this study a comparison between the performance of this rapid molecular test for RSV infection with RT-PCR in home-dwelling older adults was performed, with relatively low viral load due to age and mild disease. Positive percentage agreement (PPA) was 90.9% (95% Confidence Interval (CI) 76.4-96.8%) and negative percentage agreement (NPA) was 99.7% (95% CI 99.0-99.9%) compared to RT-PCR. All discordant results had low RSV viral titers, showing test performance is possibly dependent on viral load. Another explanation could be the use of different viral transport media for RT-PCR and Xpert® Xpress Flu/RSV. M4RT was used for RT-PCR analysis and was stored at -80°C until testing. For analysis with Xpert® Xpress we used UTM viral transport medium and was tested the same day. However, to our knowledge, there is no literature on viral transport media affecting viral load. An overview of different RSV diagnostics is shown in Table 1 and Figure 3.

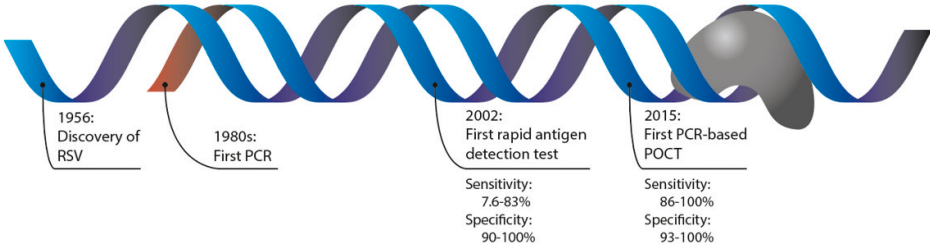


Figure 3. RSV diagnostics over the years. PCR=polymerase chain reaction. POCT=point-of-care test. RSV=respiratory syncytial virus.

Table 1. Four main diagnostic strategies for RSV [10].

Test	Turnaround time	Advantages	Disadvantages	Costs
Cell culture	3 – 7 days	Excellent specificity	Limited sensitivity	Moderate
NAAT (RT-PCR)	Hours	Excellent performance	Need high-level facilities and expertise, expensive	High
NAAT (POCT)	15 min – 1 hour	Excellent performance and fast		Moderate
Immunofluorescence	~1 h		Limited sensitivity, need expertise	High
RADT	Minutes	Fast, easy to use and small	Limited sensitivity	Low

4. Factors affecting test performance

As mentioned before, point-of-care tests (POCT) are generally specific, however sensitivity vary often resulting in a high percentage of false-negative result of the test.

Many factors affect test performance of RSV diagnostics, which can be divided into three important categories: technical factors, host factors, and clinical circumstances [19].

4.1 Technical factors

One of the most important factors in accuracy of POCTs is analytic sensitivity, determining the amount of false-negative results. It is defined by the threshold of detection, also called limit of detection. The limit of detection is the minimal amount of virus that it is needed to be detected by the test (Figure 4). This is generally expressed as tissue culture infectious dose ($TCID_{50}$/mL), which is the amount of virus required to produce a cytopathic effect in 50% of inoculated tissue culture cells. For example, reported limit of detection for IDNOW RSV assay is 5.82×10^2 $TCID_{50}$/mL and 6.0×10^1 $TCID_{50}$/mL for RSV A and B respectively. In addition, analytic sensitivity also depends on which probes or strains are used for the assay. Analytic sensitivity is the major difference between POCTs, i.e. RADT versus molecular-based POCTs. Hence, when it is important to rule out an RSV infection a test with high analytic sensitivity is needed. Tests with lower analytic sensitivity can be used when high loads are expected and false-negative test results are not critical, i.e. used as a companion diagnostic during a trial.

RSV POCTs have an excellent analytic specificity, ranging from 90 to 100% [10,17]. This demonstrates that these tests are accurate enough to guide patient management based on a positive test result. To determine the analytic specificity of POCTs, the assay is tested on a broad panel of respiratory pathogens that may be present in the oral and nasal cavity.

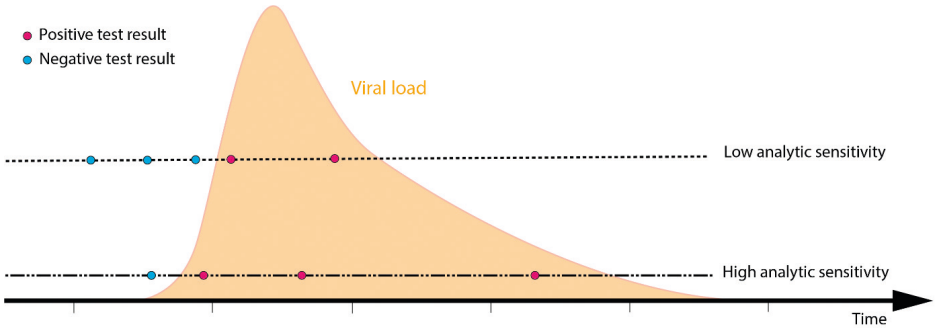


Figure 4. Analytic sensitivity
Viral load over time is shown. The two horizontal lines represent a low and a high analytic sensitive assay. Blue dots are negative tests result, the red dots are positive test results.

4.2 Host factors

Host factors affect test performance by inherently affecting viral load. The higher the viral load, the less false-negatives a test will give. Tests with a high analytic sensitivity, like PCR, are less sensitive for lower viral loads, in contrast, rapid antigen detection tests with a lower analytic sensitivity will only detect RSV if viral load is above the limit of detection. Host factors affecting viral load can be age, disease severity, and timing and method of sample collection.

4.2.1 Age

Age is an important modifier of POCT performance, with higher sensitivity in children compared to adults in a meta-analysis of the use of RADTs [19,31]. Due to partial prior immunity, the viral titers during RSV infection decreases with age, thereby lowering sensitivity of POCT performance. A decrease in viral load was found by the increase of age in children aged 0 to 24 months in prospective study of previously healthy children [32]. Another study showed that in children aged 3 years or less, RADT sensitivity decreased with age, from 84% in the 0-5 months old group to 60% in the 24-35 months old group [18].

4.2.2 Disease severity

Disease severity is another important factor. High viral loads are associated with disease severity, resulting in higher RSV viral titers in more severe ill patients [33–35]. Thereby, mild RSV infection with symptoms as runny nose, coughing can result in poorer performance and sensitivity of POCTs, possibly due to low viral loads.

4.2.3 Moment of sampling

Delay in sampling during the course of an infection, can result in lower sensitivity, as viral load decreases over time (Figure 4) [36,37]. Viral pathogens are more likely to be detected if samples are collected soon after onset of symptoms. The highest viral load is estimated from onset of symptoms and significantly decrease after 5 days [34].

4.2.4 Immunoprophylaxis

In patients receiving immunoprophylaxis, like palivizumab and possibly in the near future Nirsevimab [7], the monoclonal antibodies could compete with the antibodies of the immunoassay used for binding the viral target protein. This could lead to false-negative RADT results and should therefore in these patients interpreted with caution or the use of molecular-based assays should be considered [17,38]. However, the exact effect and duration of receiving immunoprohylaxis on RADT results is still unclear. Immunoprophylaxis does not affect the performance of molecular-based tests, as through thermal cycling specific RSV specific nucleic acids are detected.

4.3 Clinical circumstances

4.3.1 Operator differences

For the use of most POCTs there is no specific laboratory training required, however capabilities and familiarity with the test may vary widely [17]. This was evidenced by Khanom et al. that evaluated test performance of BinaxNOW [21]. Despite the BinaxNOW package insert claims a sensitivity ranging from 77% to 98%, this study found a sensitivity of 41% compared to RT-PCR when performed by trained nurses as a point-of-care test in symptomatic children. Besides, the viral load as an explanation, they suggest an alternative explanation could be excess mucus in the samples because of sampling method. This could have prevent the antigens to react with the tests anti-RSV antibodies. However, these operator differences were not specified. For POCTs it is important that they give comparable results when used by non-laboratory personnel.

4.3.2 Sampling method

Sample quality also affects viral load, with nasopharyngeal swabs or aspirates known as the gold standard for sampling of respiratory specimen. Detection of viral pathogens is enhanced with the use of flocked swabs compared to Dacron polyester or rayon swabs [39]. Flocked swabs shown comparable sensitivity to nasopharyngeal

aspirate for detection of viral pathogens and also has the advantage of being less invasive and no need for a device for collection, making it more accessible [40–42].

Viral transport media and handling of the sample could also play an important role in preserving the viral pathogen. The viral transport medium used, should be approved by the manufacturer of the POCT. The viral transport medium should be stored and transported at low temperatures [39]. Further studies need to be performed to investigate the effect of temperature on different viral transport media and RSV viral load.

4.3.3 Disease prevalence

Test performance is also affected by the prevalence of the disease. This means that there is (in case of RSV) a seasonal influence, as a higher prevalence will lead to a higher positive predictive value.

5. Advantages of POCTs

Rapid RSV test results can improve patient management, allow early infection control measures, and decrease average length of hospital stay. Rapid RSV testing can enhance antimicrobial stewardship, as it prevents empiric antibiotic treatment or enables discontinuation is it was already started [43–46]. POC testing may also reduce laboratory utilisation, and ancillary tests [47,48]. Due to the short turnaround time of POCTs, clinicians can minimise the diagnostic work-up, eliminating the necessity of additional investigations such as e.g. x-ray, blood culture and laboratory tests [47]. This reduction of ancillary testing can possibly result in more patient comfort and less associated costs. In a Hong Kong tertiary hospital, rapid diagnosis led to faster discharge from the hospitals in paediatric patients [47]. Rapid diagnosis also allows timely infection control measures, such as cohorting or isolation of RSV-positive patients [22]. This limits nosocomial transmission, as RSV is a well-recognised cause of nosocomial outbreaks [22,49–51]. Maybe the biggest advantage of POC testing in the near future will be allowing early start of treatment with antivirals. Novel therapeutics such as antivirals, immunoprophylaxis, and vaccinations are in development [11,12], with some antivirals already in phase 3 with promising results and hopefully implemented in the next years [8,52]. To be most effective they have to be administered in an early stage of the infection to prevent the development of severe disease. An urgent need for rapid diagnosis of RSV is warranted and the

need for testing in an early stage of the disease, especially testing in an early stage, for instance by the general practitioner or even by parents.

6. Limitations of POCTs

The major limitation of POCTs is their disagreement in analytic sensitivity, especially sensitivity of RADTs vary widely, which leads to a higher number of false-negative results. On the other hand, molecular-based POCTs have shown comparable performance to RT-PCR and can therefore be considered as reliable POCTs.

POCTs are not designed originally for high throughput, as most are not designed to handle multiple specimens. Most RADTs, however, are disposable and could therefore be used aside each other, testing multiple specimens at the time. Most molecular POCTs only run one sample per test, resulting in a test result every 15 to 20 minutes. Cepheid assays are performed on the GeneXpert system, which are also available in a two, four, 16, 48, or 80-module configuration, making high throughput possible.

The interpretation of the results of molecular assays may be more difficult to interpret, given that viral RNA can persist for prolonged periods and remain detectable despite their lack of clinical significance and contagiousness.

Finally, most POCTs do not differentiate between subtypes of viruses and are not able of multiplex testing. This is especially the case for RADTs. Molecular POCTs are still improving and testing for multiple pathogens will be more and more available, SARS-CoV-2/Flu/RSV for instance.

7. Cost-effectiveness

Cost-effectiveness mainly depends on costs of the assay and costs saved due to rapid diagnosis (Table 1). Also the complexity of the assay, possibility for multiplex testing, turnaround time, and the possibility of high throughput are important factors. On the other hand, costs can be saved by rapid diagnosis of RSV, resulting in faster discharge, less ancillary testing, saving of isolation cubicle time, improved antibiotic stewardship. In addition, cost-effective evaluations will vary between sites, as patient management differs between countries. BinaxNOW RSV has been evaluated on a paediatric ward in the United Kingdom by using the RADTs results to guide bed management [22]. This prospective study showed that POC testing was associated with substantial savings in the number of days that cubicles could be used for other

children. Also a study in Hong Kong demonstrated that savings were associated with benefits as shorter stay and reduction of antibiotic use by rapid diagnosis with RADT [47].

Traditionally, RADTs have been less expensive than molecular POCT systems. Cost-effectiveness of a molecular POCT for RSV is currently unknown. Despite modestly more expensive systems, it is likely that rapid diagnosis of RSV will be cost saving compared to routine clinical care [53]. Cost-effectiveness are also likely to vary between countries, accurate cost-effective evaluations are therefore needed.

CLOSING REMARKS AND FUTURE RESEARCH

In this thesis I described the dynamics of respiratory viruses in infants and the use of POCTs on RSV in infants and older adults. In this general discussion I highlighted the possibilities, advantages and disadvantages of the use of RSV POCTs.

For POCTs, high analytic sensitivity is an important feature, which has significantly improved by molecular-based assays in recent years. The use of POCTs is therefore shifting towards the use of molecular-based assays. Molecular-based POCTs testing on e.g. influenza and RSV are known, however testing on a broad panel of respiratory pathogens would be interesting for the future. The question is whether there still is a place for RADTs. The advantages of RADTs are that no machine is necessary, they are easy to use, even by non-medical persons at home, relatively cheap, and overall the fastest point-of-care test. They can also be calibrated for self-testing by patients, making diagnosis possible at the onset of respiratory symptoms [54]. However, according to previously described knowledge on RSV RADTs, they should be used with caution because of a considerable amount of false-negative results. They should only considered in specific settings where the consequences of a false-negative result are not detrimental, for example for testing on large scale during events. POCTs will be especially important for use in the community, with antivirals upcoming in the near future [11]. Rapid diagnosis in an early stage of the infection could be important, to prevent a hospitalisation due to RSV lower respiratory tract infections or respiratory failure, resulting in admission to the paediatric intensive care unit (PICU). For instance, POCTs could be used by the general practitioner or even by parents. Therefore studies evaluating accuracy and impact of POC testing in the community are important. In addition, comparative studies of different molecular POCTs in the community should be performed. The use of POCTs in the community, and primary or secondary care could shift viral testing away from traditional centralised diagnostic laboratory. Health care professionals should be prepared for this impending paradigm shift [54].

Lessons were learned from COVID-19. RADTs were used to curb the spread of the virus [55]. Rapid diagnosis of SARS-CoV-2 made timely isolation possible. Despite its moderate sensitivity, RADTs are widely used as a public health tool for screening individuals at enhanced risk of infection, to protect people who are clinically vulnerable, to ensure safe travel and resumption of schooling and social activities,

and to enable economic recovery [56]. RADTs can be used to test on large scale, fast, and cheap by the community [57,58]. They should not be used to gain access to large scale events, as false-negatives can cause super spread events. Also in hospitals, RADTs should not be used as false-negative test results can lead to nosocomial infections, and unnecessary ancillary testing and use of antibiotics. RADT should only be used as additional companion. PCR-based POCTs can be the solution for this. By using PCR-based POCTs for entrance of a big event with a large number of attendees.

In the near future of POCTs there will likely no longer be a role for RADTs. The only role for RADTs could be commercially as over-the-counter test for self-testing at home. However, the COVID-19 pandemic showed that over-the-counter (OTC) tests for SARS-CoV-2 are easy to use in the community. These tests could also become available for RSV. OTC tests could be used to track and prevent the spread of the virus, for instance at daycare or other places where people at risk for severe RSV infection are. Healthcare professionals will replace RADTs by molecular-based POCTs, especially multiplex assay combining most clinically relevant pathogens.

RSV prevention and therapeutics are within reach [59]. In this development companion diagnostics are important. RSV POCTs can be used to identify RSV in participants of trials investigating new or evaluating existing therapeutics or preventive strategies. For our RESCEU birth cohort and older adult cohort we used POCTs, making additional testing in RSV-positive participants possible. Therefore, accurate and rapid diagnosis of RSV was necessary.

The most ideal POCT is a test that is fast, reliable, easy to use and to interpret, and cheap. For the future it is important that these tests are evaluated in different populations including a wide age spectrum with different disease severities (e.g. outpatient setting). Therefore, prospectively community data is needed.

REFERENCES

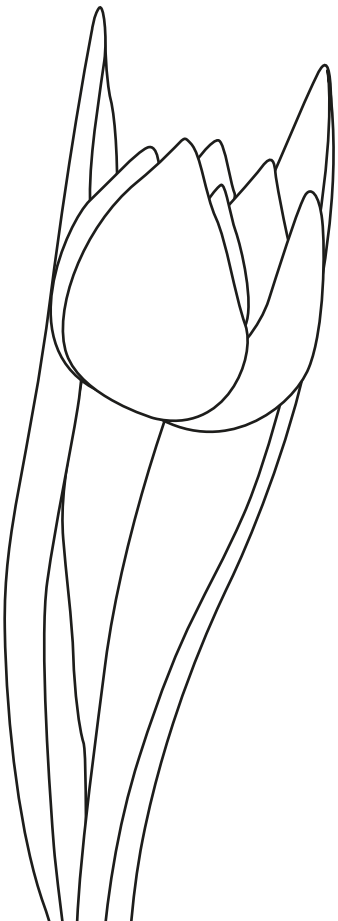
1. Shi T, McAllister DA, O'Brien KL, *et al.* Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 2017; **390**: 946–58.
2. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory Syncytial Virus Infection in Elderly and High-Risk Adults. *N Engl J Med* 2005; **352**: 1749–59.
3. Korsten K, Adriaenssens N, Coenen S, *et al.* Burden of respiratory syncytial virus infection in community-dwelling older adults in Europe (RESCEU): an international prospective cohort study. *Eur Respir J* 2021; **57**: 2002688.
4. Madhi SA, Polack FP, Piedra PA, *et al.* Respiratory Syncytial Virus Vaccination during Pregnancy and Effects in Infants. *N Engl J Med* 2020; **383**: 426–39.
5. Novavax Announces Topline RSV F Vaccine Data from Two Clinical Trials in Older Adults. 2016.
6. A Trial to Evaluate the Efficacy and Safety of RSVpreF in Infants Born to Women Vaccinated During Pregnancy. - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04424316 (accessed Aug 5, 2021).
7. Griffin MP, Yuan Y, Takas T, *et al.* Single-Dose Nirsevimab for Prevention of RSV in Preterm Infants. *N Engl J Med* 2020; **383**: 415–25.
8. A Study of Rilematovir in Infants and Children and Subsequently in Neonates Hospitalized With Acute Respiratory Tract Infection Due to Respiratory Syncytial Virus (RSV) - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04583280 (accessed Aug 5, 2021).
9. Popow-Kraupp T. Diagnosis of Respiratory Syncytial Virus Infection. *Open Microbiol J* 2011; **5**: 128–34.
10. A. Hogan C, Caya C, Papenburg J. Rapid and simple molecular tests for the detection of respiratory syncytial virus: a review. *Expert Rev Mol Diagn* 2018; **18**: 617–29.
11. Mazur NI, Martinón-Torres F, Baraldi E, *et al.* Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. *Lancet Respir Med* 2015; **3**: 888–900.
12. Cunningham S, Piedra PA, Martinon-Torres F, *et al.* Nebulised ALX-0171 for respiratory syncytial virus lower respiratory tract infection in hospitalised children: a double-blind, randomised, placebo-controlled, phase 2b trial. *Lancet Respir Med* 2021; **9**: 21–32.
13. van Houten CB, Naaktgeboren C, Buiteman BJM, *et al.* Antibiotic Overuse in Children with Respiratory Syncytial Virus Lower Respiratory Tract Infection. *Pediatr Infect Dis J* 2018; **37**: 1077–81.
14. Oosterheert JJ, van Loon AM, Schuurman R, *et al.* Impact of Rapid Detection of Viral and Atypical Bacterial Pathogens by Real-Time Polymerase Chain Reaction for Patients with Lower Respiratory Tract Infection. *Clin Infect Dis* 2005; **41**: 1438–44.
15. Alere BinaxNOW RSV Card - Alere is now Abbott. https://www.alere.com/en/home/product-details/binaxnow-rsv.html (accessed Dec 12, 2018).
16. Miernyk K, Bulkow L, Debyle C, *et al.* Performance of a rapid antigen test (Binax NOW ® RSV) for diagnosis of respiratory syncytial virus compared with real-time polymerase chain reaction in a pediatric population. *J Clin Virol* 2010; **50**: 240–3.
17. Prendergast C, Papenburg J. Rapid antigen-based testing for respiratory syncytial virus: moving diagnostics from bench to bedside? *Future Microbiol* 2013; **8**: 435–44.

18. Papenburg J, Buckeridge DL, De Serres G, Boivin G. Host and Viral Factors Affecting Clinical Performance of a Rapid Diagnostic Test for Respiratory Syncytial Virus in Hospitalized Children. *J Pediatr* 2013; **163**: 911–3.
19. Chartrand C, Tremblay N, Renaud C, Papenburg J. Diagnostic Accuracy of Rapid Antigen Detection Tests for Respiratory Syncytial Virus Infection: Systematic Review and Meta-analysis. *J Clin Microbiol* 2015; **53**: 3738–49.
20. Bruning AHL, Leeflang MMG, Vos JMBW, *et al.* Rapid Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2017; **65**: 1026–32.
21. Khanom AB, Velvin C, Hawrami K, *et al.* Performance of a nurse-led paediatric point of care service for respiratory syncytial virus testing in secondary care. *J Infect* 2011; **62**: 52–8.
22. Mills JM, Harper J, Broomfield D, Templeton KE. Rapid testing for respiratory syncytial virus in a paediatric emergency department: benefits for infection control and bed management. *J Hosp Infect* 2011; **77**: 248–51.
23. Zuurbier RP, Bont LJ, Langedijk AC, *et al.* Low Sensitivity of BinaxNOW RSV in Infants. *J Infect Dis* 2020; **222**: S640–7.
24. Ling L, Kaplan SE, Lopez JC, Stiles J, Lu X, Tang Y-W. Parallel Validation of Three Molecular Devices for Simultaneous Detection and Identification of Influenza A and B and Respiratory Syncytial Viruses. *J Clin Microbiol* 2017; **56**. DOI:10.1128/JCM.01691-17.
25. Cohen DM, Kline J, May LS, *et al.* Accurate PCR Detection of Influenza A/B and Respiratory Syncytial Viruses by Use of Cepheid Xpert Flu+RSV Xpress Assay in Point-of-Care Settings: Comparison to Prodesse ProFlu+. *J Clin Microbiol* 2017; **56**: e01237-17.
26. Popowitch EB, Miller MB. Comparison of the Xpert Flu/RSV XC and Xpress Flu/RSV Assays. *J Clin Microbiol* 2018; **56**. DOI:10.1128/JCM.00278-18.
27. Zou X, Chang K, Wang Y, *et al.* Comparison of the Cepheid Xpert Xpress Flu/RSV assay and commercial real-time PCR for the detection of influenza A and influenza B in a prospective cohort from China. *Int J Infect Dis* 2019; **80**: 92–7.
28. Ho YII, Wong AH, Lai RWM. Comparison of the Cepheid Xpert Xpress Flu/RSV Assay to in-house Flu/RSV triplex real-time RT-PCR for rapid molecular detection of Influenza A, Influenza B and Respiratory Syncytial Virus in respiratory specimens. *J Med Microbiol* 2018; **67**: 1576–80.
29. Abbott Diagnostics Scarborough Inc. ID NOW™ RSV Package Insert. 2020.
30. Product Insert Xpert Xpress Flu/RSV CE-IVD 301-7239, Rev C. 2019 https://www.cepheid.com/Package Insert Files/Xpress-Flu-RSV-US-IVD-ENGLISH-Package-Insert-301-7239-Rev. C.pdf (accessed April 28, 2020).
31. Walsh E. Respiratory Syncytial Virus Infection in Adults. *Semin Respir Crit Care Med* 2011; **32**: 423–32.
32. Brenes-Chacon H, Garcia-Mauriño C, Moore-Clingenpeel M, *et al.* Age-dependent Interactions Among Clinical Characteristics, Viral Loads and Disease Severity in Young Children With Respiratory Syncytial Virus Infection. *Pediatr Infect Dis J* 2021; **40**: 116–22.
33. Houben ML, Coenjaerts FEJ, Rossen JWA, *et al.* Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. *J Med Virol* 2010; **82**: 1266–71.
34. Garcia-Mauriño C, Moore-Clingenpeel M, Thomas J, *et al.* Viral Load Dynamics and Clinical Disease Severity in Infants with Respiratory Syncytial Virus Infection. 2017. DOI:10.1093/infdis/jiy655/5173953.

35. Uusitupa E, Waris M, Heikkinen T. Association of Viral Load With Disease Severity in Outpatient Children With Respiratory Syncytial Virus Infection. *J Infect Dis* 2020; **222**: 298–304.
36. Hall CB, Douglas RG, Geiman JM. Respiratory syncytial virus infections in infants: Quantitation and duration of shedding. *J Pediatr* 1976; **89**: 11–5.
37. Takeyama A, Hashimoto K, Sato M, Kawashima R, Kawasaki Y, Hosoya M. Respiratory syncytial virus shedding by children hospitalized with lower respiratory tract infection. *J Med Virol* 2016; **88**: 938–46.
38. Abbott Diagnostics Scarborough I. BinaxNOW™ RSV Package Insert. 2020.
39. Druce J, Garcia K, Tran T, Papadakis G, Birch C. Evaluation of Swabs, Transport Media, and Specimen Transport Conditions for Optimal Detection of Viruses by PCR. *J Clin Microbiol* 2012; **50**: 1064–5.
40. Debyle C, Bulkow L, Miernyk K, *et al.* Comparison of nasopharyngeal flocked swabs and nasopharyngeal wash collection methods for respiratory virus detection in hospitalized children using real-time polymerase chain reaction. *J Virol Methods* 2012; **185**: 89–93.
41. Munywoki PK, Hamid F, Mutunga M, Welch S, Cane P, Nokes DJ. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real-time PCR using nasopharyngeal flocked swabs. *J Clin Microbiol* 2011; **49**: 3365–7.
42. Chan KH, Peiris JSM, Lim W, Nicholls JM, Chiu SS. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. *J Clin Virol* 2008; **42**: 65–9.
43. Bruning AHL, de Kruijf WB, van Weert HCPM, *et al.* Diagnostic performance and clinical feasibility of a point-of-care test for respiratory viral infections in primary health care. *Fam Pract* 2017; **34**: 558–63.
44. Byington CL, Castillo H, Gerber K, *et al.* The Effect of Rapid Respiratory Viral Diagnostic Testing on Antibiotic Use in a Children's Hospital. *Arch Pediatr Adolesc Med* 2002; **156**: 1230.
45. Adcock PM, Stout GG, Hauck MA, Marshall GS. Effect of rapid viral diagnosis on the management of children hospitalized with lower respiratory tract infection. *Pediatr Infect Dis J* 1997; **16**: 842–6.
46. Ferronato A, Gilio A, Ferraro A, Paulis M, Vieira S. Etiological diagnosis reduces the use of antibiotics in infants with bronchiolitis. *Clinics* 2012; **67**: 1007–11.
47. Woo PC, Chiu SS, Seto WH, Peiris M. Cost-effectiveness of rapid diagnosis of viral respiratory tract infections in pediatric patients. *J Clin Microbiol* 1997; **35**: 1579–81.
48. Barenfanger J, Drake C, Leon N, Mueller T, Troutt T. Clinical and Financial Benefits of Rapid Detection of Respiratory Viruses: an Outcomes Study. *J Clin Microbiol* 2000; **38**: 2824–8.
49. Berger A, Obwegeser E, Aberle SW, Langgartner M, Popow-Kraupp T. NOSOCOMIAL TRANSMISSION OF RESPIRATORY SYNCYTIAL VIRUS IN NEONATAL INTENSIVE CARE AND INTERMEDIATE CARE UNITS. *Pediatr Infect Dis J* 2010; **29**: 669–70.
50. Krasinski K, LaCouture R, Holzman RS, Waithe E, Bonk S, Hanna B. Screening for respiratory syncytial virus and assignment to a cohort at admission to reduce nosocomial transmission. *J Pediatr* 1990; **116**: 894–8.
51. Madge P, Paton JY, McColl JH, Mackie PLK. Prospective controlled study of four infection-control procedures to prevent nosocomial infection with respiratory syncytial virus. *Lancet* 1992; **340**: 1079–83.
52. Jorquera PA, Tripp RA. Respiratory syncytial virus: prospects for new and emerging therapeutics. *Expert Rev Respir Med* 2017; **11**: 609–15.

53. Brendish NJ, Malachira AK, Beard KR, Ewings S, Clark TW. Impact of turnaround time on outcome with point-of-care testing for respiratory viruses: a post hoc analysis from a randomised controlled trial. *Eur Respir J* 2018; **52**: 1800555.
54. Basile K, Kok J, Dwyer DE. Point-of-care diagnostics for respiratory viral infections. Expert Rev. Mol. Diagn. 2018; **18**: 75–83.
55. To KKW, Sridhar S, Chiu KHY, *et al.* Lessons learned 1 year after SARS-CoV-2 emergence leading to COVID-19 pandemic. *Emerg Microbes Infect* 2021; **10**: 507.
56. Peeling RW, Heymann DL, Teo Y-Y, Garcia PJ. Diagnostics for COVID-19: moving from pandemic response to control. *Lancet* 2022; **399**: 757–68.
57. Bootsma M, Kretzschmar M, Rozhnova G, Heesterbeek J, Kluytmans J, Bonten M. Regular universal screening for SARS-CoV-2 infection may not allow reopening of society after controlling a pandemic wave. *medRxiv* 2020; : 2020.11.18.20233122.
58. Larremore DB, Wilder B, Lester E, *et al.* Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. *Sci Adv* 2021; **7**: eabd5393.
59. Mazur NI, Terstappen J, Baral R, *et al.* Respiratory syncytial virus prevention within reach: the vaccine and monoclonal antibody landscape. *Lancet Infect Dis* 2022; **0**. DOI:10.1016/S1473-3099(22)00291-2/ATTACHMENT/D365FE1B-D4A5-4F10-AC00-3F841DF03B7A/MMC1.PDF.

Sweet wonderful you
You make me happy with the things you do
(Fleetwood Mac – You Make Loving Fun)



Appendices

NEDERLANDSE SAMENVATTING

Luchtweginfecties

Luchtweginfecties (LWI's) behoren tot de meest voorkomende aandoeningen bij kinderen. Deze kunnen leiden tot lagere luchtweginfecties (LLWI), zoals longontsteking en bronchiolitis. De mortaliteit van LWI's wordt geschat op 921.000 gevallen in 2015. LWI's zijn de tweede belangrijkste doodsoorzaak bij jonge kinderen (<5 jaar), met de grootste ziektelast bij zuigelingen (<1 jaar). 99% van de sterfte vindt plaats in ontwikkelingslanden. Door verbeterde sociaaleconomische omstandigheden, meer nadruk op preventieve interventies en verbeterde toegang tot en kwaliteit van de gezondheidszorg neemt de wereldwijde incidentie en mortaliteit van LWI's bij jonge kinderen af. Desalniettemin vormen LWI's nog steeds een aanzienlijke belasting voor de gezondheidszorg, ook in ontwikkelde landen.

De meest voorkomende oorzaak van LWI's zijn (seizoensgebonden) verkoudheidsvirussen, ook wel respiratoire virussen. Bekende respiratoire virussen zijn respiratoir syncytieel virus (RSV), influenzavirus en rhinovirus. Deze virussen zijn de belangrijkste oorzaken van ziekenhuisopnames, morbiditeit en mortaliteit bij kinderen. Dit is vooral hoog in risicogroepen zoals te vroeg geboren kinderen, kinderen met een aangeboren hart- of longziekte of kinderen met het syndroom van Down. RSV zorgt voor het merendeel van de ernstige luchtweginfecties bij jonge kinderen. Respiratoire virussen kunnen echter ook worden gevonden bij kinderen met milde symptomen. Om meer inzicht te krijgen in kinderen met een respiratoir virus die zich presenteren met milde symptomen zijn geboortecohortstudies nodig. Op die manier kunnen alle gradaties van ziekte ernst worden onderzocht.

De meeste onderzoeken zijn grotendeels gedaan met gehospitaliseerde patiënten, waarbij de nadruk ligt op de ernstigere gevallen. Daarmee laten deze onderzoeken alleen het topje van de ijsberg zien. Om het volledige epidemiologische bereik van LWI's bij zuigelingen te begrijpen, is een geboortecohort nodig. Zo kunnen zelfs virussen worden gevonden die geen symptomen geven. Geboortecohortstudies zijn ook nodig om kennis en daarmee preventieve interventies te verbeteren. De veel grotere groep van niet-ernstige patiënten zorgt namelijk voor een substantiële sociaaleconomische last door gebruik van de gezondheidszorg, onnodig antibiotica gebruik en school- en ouderlijk werkverzuim.

Diagnostiek

Momenteel is de gouden standaard voor de diagnose van RSV een laboratorium test genaamd reverse transcriptase polymerase chain reaction (RT-PCR). Deze techniek heeft een hoge gevoeligheid en specificiteit, maar is tijdrovend, afhankelijk van getraind laboratoriumpersoneel en heeft een doorlooptijd van 24-48 uur voordat de resultaten beschikbaar zijn voor klinische teams. Dit kan de klinische waarde sterk verminderen. Een betrouwbare snelle diagnostische test kan veel voordelen hebben. Zo zou het onnodige voorschrijven van antibiotica kunnen verminderen en zouden gehospitaliseerde patiënten met een infectie door RSV geclusterd kunnen worden, zodat zij geen andere patiënten kunnen besmetten. Daarnaast kan een sneltest belangrijk zijn voor het gebruik van nieuwe antivirale RSV medicijnen, waarbij het belangrijk is om in een vroeg stadium van de infectie te starten met medicatie.

De afgelopen jaren zijn er verschillende sneltesten of point-of-care testen (POCT's) ontwikkeld om RSV te detecteren, zoals snelle antigeentesten (ADT's) en moleculaire testen. Er is een reeks POCT's beschikbaar die al in de klinische praktijk worden gebruikt, omdat ze snel, gebruiksvriendelijk en vaak goedkoper zijn dan een standaard RT-PCR. RSV ADT's zijn POCT's met een hoge specificiteit, maar een breed bereik in gevoeligheid, wat deels afhankelijk is van de hoeveelheid virus deeltjes. Deze testen zijn vergelijkbaar met de corona sneltesten zoals wij die heden kennen. De meest gebruikte techniek bij ADT's zijn laterale-flow immunochromatografische testen. Deze kunnen worden vergeleken met een zwangerschapstest. Twee recente meta-analyses toonden een gevoeligheid van 81% (95% betrouwbaarheidsinterval (BI), 78-84%) en 75,9% (95% BI, 73,1-78,5%) voor RSV ADT's bij kinderen in vergelijking met RT-PCR. Er is een grote heterogeniteit in deze onderzoeken, waarbij het onderzoek vaak wordt gesponsord door de fabrikant van de tests. Bovendien zijn veel onderzoeken retrospectief en bestaat de populatie uit kinderen in het ziekenhuis, waarbij de diagnostiek niet meteen ter plekke wordt uitgevoerd. Hierdoor varieert de gevoeligheid van individuele onderzoeken aanzienlijk van 41,2% tot 83%.

PCR gebaseerde moleculaire sneltesten zijn ook beschikbaar en worden in de klinische praktijk gebruikt omdat ze snel, gemakkelijk te gebruiken door niet-laboratoriumpersoneel en vaak goedkoper in vergelijking met routinematige RT-PCR zijn. De doorlooptijd van de meeste moleculaire POCT's is minder dan een uur. Onderzoeken laten een hoge gevoeligheid en specificiteit zien, maar mogelijk wordt de testnauwkeurigheid wel overschat. Deze onderzoeken werden namelijk uitgevoerd

bij gehospitaliseerde patiënten met overgebleven monsters en gedeeltelijk bij kinderen met voorspelbaar hoog aantal virus deeltjes. Bovendien vond er vaker wel dan niet sponsoring door de fabrikant plaats en waren de patiënten groepen relatief klein. Om die reden is het ook voor deze sneltesten belangrijk om de testprestaties in geboortecohorten te evalueren, waarbij verschillende gradaties van ziekte ernst worden meegenomen in het onderzoek.

Deel één: RSV sneltesten

In **deel één** van het proefschrift beschreef ik het gebruik van verschillende sneltesten op RSV bij zuigelingen en bij oudere volwassenen. De afgelopen jaren zijn er verschillende sneltesten ontwikkeld om RSV op te sporen. Onze onderzoeken werden uitgevoerd binnen ons internationale RESCEU-geboortecohort en oudere volwassen cohort waarin het primaire doel was om de last van RSV te bepalen. Vanwege het prospectieve karakter van deze cohortstudies waren we in staat om de nauwkeurigheid te onderzoeken van verschillende sneltesten in een bepaalde groep, met verschillende niveaus van ernst van RSV-infecties.

In **Hoofdstuk 2** werden de prestaties van de antigeendetectietest BinaxNOW® RSV (BN) onderzocht. Neuswatten van zuigelingen met luchtweginfecties met verschillende gradaties van ziekte ernst werden geanalyseerd met BN in vergelijking met een moleculair diagnosticum. In totaal werden 162 neuswatten van 148 kinderen bestudeerd. Er werd een lage gevoeligheid gevonden van de antigeentest BN voor het aantonen van RSV bij zuigelingen, met een gevoeligheid van 7,6% (95% BI, 3,3-16,5%), de specificiteit was 100% (95% BI 96,2-100%). De gevoeligheid was iets hoger in de subgroep van baby's die op een kinder intensive care waren opgenomen in vergelijking met minder ernstig zieke baby's (22,2% versus 5,3%), hoewel dit verschil niet statistisch significant was (p=0,134) en de gevoeligheid laag bleef.

De prestaties van een moleculaire sneltest bij oudere volwassenen werden beoordeeld in **Hoofdstuk 3**. Neuswatten van deelnemers aan RESCEU's ouderen cohortstudie werden verzameld telkens wanneer ze een luchtweginfectie doormaakten. De prestaties van de Xpert® Xpress Flu/RSV test werden geëvalueerd om RSV infectie te diagnosticeren bij thuiswonende oudere volwassenen (≥60 jaar) met luchtweginfecties met verschillende gradaties van ziekte ernst. De prestaties van Xpert® Xpress Flu/RSV, vergeleken met de routinematige RT-PCR, zijn hoog voor de detectie van RSV bij thuiswonende oudere volwassenen. In alle gevallen waarvan de

resultaten niet overeenkwamen met de referentietest was de virale load laag. Het positieve percentage van overeenstemming (PPA) was 90,9% (95% BI 76,4-96,8%) en het negatieve percentage van overeenstemming (NPA) was 99,7% (95% BI 99,0-99,9%). Het positieve percentage van overeenstemming werd gebruikt als uitkomst in plaats van als gevoeligheid, om zo de overeenkomst tussen twee nauwkeurige tests aan te tonen. De test is snel en gemakkelijk te gebruiken en kan daarom het beleid en de uitkomsten voor de patiënt verbeteren.

Deel twee: verkoudheidsvirussen bij zuigelingen

In **deel twee** van dit proefschrift besprak ik de ziektelast en dynamiek van verkoudheidsvirussen bij zuigelingen. De resultaten zijn gebaseerd op een ziekenhuiscohort en twee verschillende geboortecohortstudies: het Nederlandse MUIS geboortecohort en de internationale RESCEU studie bij zuigelingen. Beide geboortecohortstudies, MUIS en het Nederlandse deel van RESCEU, werden uitgevoerd in het Spaarne Gasthuis. Binnen deze cohorten konden we verschillende gradaties van ziekte ernst onderzoeken.

In **Hoofdstuk 4** onderzochten we het voorkomen van respiratoire virussen bij zuigelingen tijdens het eerste levensjaar. Er werden 1.304 neuswatten afgenomen uit 11 opeenvolgende reguliere afnamemomenten en tijdens een luchtweginfectie. Rhinovirus (RV) was negatief geassocieerd met het hebben van een luchtweginfectie (aangepaste odds ratio 0,41 [95% BI 0,18-0,92]). Het humaan metapneumovirus, RSV, para-influenza (PIV) 2 en 4 en het humaan coronavirus (HCoV) HKU1 waren positief geassocieerd met een luchtweginfectie. Een symptoomloze infectie door RV in de eerste maanden van het leven was echter geassocieerd met een verhoogde vatbaarheid voor en recidief van luchtweginfecties later in het eerste levensjaar (Kaplan-Meier overlevingsanalyse: p=0,022). Over het algemeen worden verkoudheidsvirussen vaak gedetecteerd bij zuigelingen en gaan ze zelden met symptomen gepaard.

De methoden van het prospectieve internationale RESCEU geboortecohort werden beschreven in **Hoofdstuk 5**. Deze multicenter studie had als doel om gedurende drie opeenvolgende jaren 10.000 gezonde op tijd geboren zuigelingen te includeren, waaronder een cohort van 1.000 baby's die actief werden gevolgd. In dit extra cohort werd tijdens alle luchtweginfecties in het RSV-seizoen een neuswat afgenomen om te testen op RSV. De primaire uitkomstmaten waren de incidentie van RSV-geassocieerde

LWI, LWI waarvoor een doktersbezoek nodig was en ziekenhuisopname in het eerste levensjaar. Dit zal belangrijke informatie opleveren in de kennis over de ziektelast van RSV bij gezonde zuigelingen en de besluitvorming voor de implementatie van nieuwe preventiestrategieën ondersteunen.

De resultaten van bovenstaande studie zijn weergegeven in **Hoofdstuk 6**. In totaal werden 9.154 zuigelingen, geboren tussen juli 2017 en april 2020, gedurende het eerste levensjaar gevolgd, waarvan er 993 deelnamen aan het actieve deel van de studie. De incidentie van ziekenhuisopnames door RSV in het totale cohort was 1,8% (95% BI 1,6-2,1). Ongeveer de helft van de ziekenhuisopnames voor luchtweginfecties in het eerste levensjaar was geassocieerd met RSV. De meerderheid (57,9%) van ziekenhuisopnames door RSV vond plaats bij kinderen onder de 3 maanden oud. De incidentie van RSV infectie en RSV infectie onder medisch toezicht in het actieve surveillancecohort was respectievelijk 26,2% (95% BI 24,0-28,6) en 14,1% (95% BI 12,3-16,0). Immunisatie van zwangere vrouwen of gezonde op tijd geboren zuigelingen tijdens hun eerste winterseizoen kan een aanzienlijke impact hebben op de zorglast veroorzaakt door RSV infecties.

De ziektelast van het griepvirus (influenza) en RSV bij zuigelingen van het Nederlandse deel van RESCEU werd besproken in **Hoofdstuk 7**. Een totale incidentie van 3,2% (6/187) voor influenza luchtweginfecties en 35,8% (67/187) voor RSV LWI's werd gevonden in het geboortecohort. Het ziekenhuiscohort werd uitgevoerd in het Spaarne Gasthuis. In dit cohort werd bij 7,6% (23/304) van de gehospitaliseerde zuigelingen met een luchtweginfectie influenza vastgesteld. Bij RSV was dit 49,3% (150/304). RSV was verantwoordelijk voor het grootste aantal LWI's bij zowel niet-gehospitaliseerde als gehospitaliseerde baby's, vooral tijdens de eerste levensmaanden. De incidentie van influenza was laag in vergelijking met RSV. Deze bevindingen in dit onderzoek laten zien dat de meeste nadruk moet liggen op preventiestrategieën tegen RSV, vooral in de eerste levensmaanden.

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LIST OF PUBLICATIONS

In this thesis:

Zuurbier, R. P., Bont, L. J., Langedijk, A. C., Hamer, M., Korsten, K., Drysdale, S. B., … & Wildenbeest, J. G.. Low sensitivity of BinaxNOW RSV in infants. *The Journal of infectious diseases, 222*(Supplement 7), S640-S647 (2020).

Zuurbier, R. P., Korsten, K., Verheij, T.J.M., … & van Houten M.A., Bont, L.J., Wildenbeest, J. G.. Performance Assessment of a Rapid Molecular Respiratory Syncytial Virus Point-of-Care Test: A Prospective Community Study in Older Adults. *The Journal of Infectious Diseases,* 226(Suppl 1):S63-S70 (2022).

Zuurbier, R. P., Bogaert, D., de Steenhuijsen Piters, W.A.A., Arp, K., Chu, M.L.J.N, Sanders, E.A.M., van Houten, M.A.. Asymptomatic Viral Presence in Early Life Precedes Recurrence of Respiratory Tract Infections. *The Pediatric Infectious Disease Journal,* 42(1):59-65 (2022).

Wildenbeest J.G., **Zuurbier, R. P*.**, Billard, M.N*., van Houten M.A., Korsten, K., ..., Bont L.J. The burden of respiratory syncytial virus in healthy termborn infants in Europe: a prospective birth cohort study. *The Lancet. Respiratory medicine*, S2213-2600(22)00414-3 (2022).

Wildenbeest, J. G., **Zuurbier, R. P.**, Korsten, K., van Houten, M. A., Billard, M. N., Derksen-Lazet, N., ... & Bont, L. J.. Respiratory Syncytial Virus Consortium in Europe (RESCEU) Birth Cohort Study: Defining the Burden of Infant Respiratory Syncytial Virus Disease in Europe. *The Journal of Infectious Diseases,* 222(Supplement_7), S606-S612 (2020).

Zuurbier, R. P., Bont, L. J., Korsten, K., Billard, M.N., de Groot P.C.M., ... & Wildenbeest, J. G., van Houten, M.A.. The burden of respiratory syncytial virus and influenza associated acute respiratory tract infection in infants. Unpublished

Not in this thesis

Bosch, S., van Gaal, N., **Zuurbier, R. P.**, Covington, J. A., Wicaksono, A. N., Biezeveld, M. H., ... & de Meij, T. G.. Differentiation between pediatric irritable bowel syndrome and inflammatory bowel disease based on fecal scent: proof of principle study. *Inflammatory bowel diseases*, *24*(11), 2468-2475 (2018).

Zuurbier, R. P., Herpers B.L., van Houten, M. A.. Geen antistoffen bij milde klachten bij COVID-19: gezinscasusserie. *Tijdsschrift voor Infectieziekten, 15*(COVID-19-SPECIAL), 21-7 (2020).

* These authors contributed equally

PHD PORTFOLIO

Courses	Year	ECT
General		
eBROK	2017	1.5
Introductory Biostatistics for Researchers	2019	4.5
Writing a scientific paper	2019	1.5
Time management & planning	2020	0.3
Effective oral presentation	2018	0.3
Adobe InDesign	2020	0.6
Adobe Illustrator	2021	0.6
Infection & Immunity courses		
Advanced infection biology	2019	1.5
Advanced immunology	2021	1.5
Scientific presentations and (inter)national conferences		
ReSViNET Malaga	2017	0.9
General assembly meeting RESCEU II – presentation and poster	2018	0.9
Wetenschap in Beeld symposium Spaarne Gasthuis - presentation	2018	0.3
ReSViNET Ghana – poster	2019	0.9
General assembly meeting RESCEU III – presentation and poster	2019	0.9
Amsterdam Kindersymposium	2019	0.3
RIVM RVP onderzoeksdag	2019	0.3
Amsterdam Kindersymposium – presentation	2020	0.3
European Society for Paediatric Infectious Diseases – poster discussion	2020	0.9
I&I 25th symposium	2020	0.3
Meetings		
RSV group meetings	2017-2021	2
Lab meetings (Bogaert group)	2018-2020	1
Journal club Spaarne Gasthuis	2020-2021	0.5
Supervising		
SUMMA research internship – J.J. Kroese-van Dieren	2018	0.5
Master internship medicine (wetenschappelijke stage) - A.E. de Jong	2020	0.5

ABOUT THE AUTHOR

Roy (Raymond Petrus) Zuurbier was born on Sunday the 22nd of March, 1992 in Hoorn, the Netherlands. He grew up in Grootebroek with his parents and sister Maudy. He went to secondary school (Gymnasium) at the Martinus College in Grootebroek. In 2007 his father passed away. After graduating in 2010, he studied medicine at the Vrije Universiteit Amsterdam. He graduated as a medical doctor in 2016, after which he worked as junior doctor (ANIOS) at the Department of Paediatrics of Spaarne Gasthuis, Hoofddorp. In May 2017 he started his PhD program under the supervision of prof. dr. L.J. Bont, dr. J.G. Wildenbeest, and dr. M.A. van Houten at the Spaarne Gasthuis and Wilhelmina Children's Hospital in Utrecht. Results of this research are presented in this thesis. In 2021 he started as a junior doctor at the Department of Paediatrics at Tergooi MC, Blaricum. In April 2023 he started as a paediatric resident (AIOS) at the Amsterdam University Medical Center under the supervision of dr. D.K. Bosman and dr. H.M.A. de Bie.

Roy is happily married with Annabel. They live together with their son Sep (2023) in Weesp.